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Capacity audience of scientists from all parts of United States and from Canada listen intently to one of speakers at AMIF Eleventh Annual Research Conference, held on March 26 and 27, 1959. Auditorium is in Oriental Institute Museum at The University of Chicago.

Instituted in 1949 under auspices of the American Meat Institute, the Research Conference now is conducted annually under the sponsorship of the Research Advisory Council of the American Meat Institute Foundation. Par-

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I

RECENT RESEARCH ON HUMAN PROTEIN REQUIREMENTS AND THE AMINO ACID SUPPLEMENTATION OF FOODS

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Purdue University
Lafayette, Indiana

Physiologists, chemists and nutritionists have been grappling with the problem of man's protein requirement for well over a half century. Research during the past decade has not answered all of the questions, but it has provided a better understanding of the basic problem. This problem is two-fold: (a) to determine the minimum protein intake which will prevent protein deficiency diseases, such as kwashiorkor, in countries with limited supplies of protein, and (b) to determine the minimum protein intake compatible with the best state of health in a population which can afford to eat adequate quantities of protein.

In order to proceed in a logical manner, protein requirements will be discussed first in terms of a protein of high digestibility and nutritive quality, such as whole egg protein, and later other protein sources will be compared with the reference protein.

Table 1 summarizes the minimum average protein requirements at different ages for proteins of high biological value such as egg protein. These estimates were presented by Sebrell (1) and are based mainly on the conclusions of a board of nutrition experts (2).

TABLE 1

Estimated Minimum Daily Protein Intakes to Prevent Protein Deficiency Disease

Age (years)	Protein Intake (gm./kg. B.W.)	Age (years)	Protein Intake (gm./kg. B.W.)
0	(1.6*)	12	0.7
9/12	1.4	15	0.8
2	1.2	16	0.7
4	0.9	17	0.55
6	0.75	18	0.45
8	0.7	21	0.35
10	0.7		

* Based on human milk (3). Other values from Sebrell (1), assuming protein to be egg protein or equivalent.

Inspection of Table 1 reveals that the highest daily intake, 1.6 gm. per kg. of body weight, is needed during the first nine months of life. At 2 years of age the level has dropped to 1.2 gm. and at 8 years of age to 0.7 gm. where it re-

mains fairly constant until the late teens. It then gradually decreases to 0.35 gm. at maturity. This low level of 0.35 gm. is apparently maintained throughout adult life, except during periods of disease, pregnancy or lactation. György (3) believes that the protein requirement of the infant is 1.6 gm. per kg. because human milk provides this level.

In all discussions of protein requirements, it must be borne in mind that calorie requirements must also be satisfied. If the caloric intake is reduced below a critical level, protein requirements increase as proteins are burned in place of carbohydrate or fat (4). The minimum caloric intakes recommended by the National Research Council (5) are 120 calories per kg. body weight at birth, decreasing at maturity to 45 calories for the "reference" male and 40 calories for the "reference" female.

Returning to the values in Table 1, it is the opinion of the writer that these are the best estimates we now have of the minimum intakes which will prevent protein deficiency diseases such as kwashiorkor. According to Scrimshaw and coworkers (6) the word kwashiorkor is used to describe "a syndrome which includes retarded growth and maturation, edema, alterations in the skin and hair, apathy, anorexia, diarrhea and a variety of accompanying biochemical and physiological changes." These workers (6) state that "sufficient milk or other protein of animal origin, given to young children whenever the supply of mother's milk becomes inadequate for their needs, would effectively prevent the occurrence of kwashiorkor. Unfortunately, animal protein is costly and in short supply in most areas where the syndrome is a problem."

Research on this protein deficiency disease is in progress in many technically underdeveloped countries. Active workers in the field who have published reports recently on the prevention and treatment of kwashiorkor are Scrimshaw and coworkers of Guatemala (6,7), Gómez and coworkers of Mexico (8), Sénécal of French West Africa (9), Garrow of the British West Indies (10), and Brock and Hansen of South Africa (11). Scrimshaw and coworkers (6) have developed a native vegetable mixture as a replacement for milk and other animal protein sources. The mixture (Table 2) consists of dried corn masa, sesame meal, cottonseed press cake, Torula yeast, and kikuyu-leaf meal. In nitrogen balance experiments with 12 children, the vegetable mixture, fed as a hot sweetened gruel, had a biological value essentially equivalent to that of milk. Three of the children were placed on the mixture upon admission to the hospital with acute kwashiorkor, and made satisfactory recoveries.

TABLE 2

Composition of INCAP Vegetable Mixture 8*

Ingredient	Per Cent	Ingredient	Per Cent
Dried corn masa	50	Protein	25.1
Sesame meal	35	Fat	13.7
Cottonseed press cake	9	Carbohydrate	46.3
Torula yeast	3	Crude Fiber	3.2
Kikuyu-leaf meal	3	Ash	6.6

* From Scrimshaw et al. (6).

The use of similar mixtures in other technically underdeveloped areas of the world would, if accepted by the natives, help to prevent kwashiorkor during the critical preschool years of life when the protein requirements are high. If such mixtures were well accepted by all age groups, consumption of protein would then rise above the minimum levels shown in Table 1, and a

more vigorous, healthy population would result.

In our discussion of man's protein requirement, we have looked at only one side of the coin—how to wipe out severe protein deficiency disease. The other equally important side is to determine the level of protein needed for the best state of health in a prosperous population such as ours. The spread between minimum requirement to prevent frank protein deficiency disease, and the requirement for the best state of health, is probably three-fold or more at every age level shown in Table 1. This spread is most readily explained on the basis of the need for full "labile protein" stores in the body.

Allison (12) defines labile proteins as "blood and tissue proteins that increase or decrease in quantity as the dietary nitrogen is raised or lowered respectively." Proteins that readily qualify as labile are those of the liver, pancreas and intestinal wall. Suboptimal protein nutrition causes large reductions in the enzymes and proenzymes elaborated by these organs, which is apparently responsible for the parallel reduction in blood serum enzyme levels (6,13).

Tracer experiments have demonstrated that the proteins of the internal organs and glands—spleen, liver, pancreas, kidney, intestines and testes have high turnover rates for protein, whereas those of the skin, muscle, bone, lung and brain appear to have low turnover rates. However, Lajtha and coworkers (14) have recently shown that even muscle and brain tissue have fractions of high turnover rate, comparable in half-life to liver proteins of high turnover. From this one may conclude that labile proteins are present in all body tissues, even those made up largely of structural-type proteins.

Allison (15) has determined the minimum protein requirements of dogs, comparing nitrogen-depleted animals with animals having full nitrogen stores. With whole egg protein, the requirement was 0.4 gm. per day per kg. of body weight in the depleted dog, compared with 1.4 gm. in the dog with full nitrogen stores. When wheat gluten was used as the protein source, the depleted dog required 0.6 gm., and the dog with full nitrogen stores required 2.8 gm. Values for beef and casein were intermediate between egg protein and wheat gluten. Thus, to obtain full stores, protein intake of the depleted dog had to be increased three to four-fold, the protein of lowest nutritive value requiring the greatest increase.

If protein-depleted dogs were fed a high quality protein, the labile protein stores increased and the animals retained nitrogen. However, the curve of nitrogen storage followed the law of diminishing returns, the per cent of nitrogen retention of absorbed nitrogen falling off as the dietary nitrogen increased. The exponential shape of the nitrogen retention curve (15) is probably due to the gradual filling of the labile protein stores, the repletion producing higher levels of blood amino acids and liver enzymes, and causing increased oxidative destruction of the amino acids.

If full labile protein stores are to be maintained in humans, the dog studies of Allison suggest that the protein intake levels in Table 1 should be multiplied by a factor of three for egg protein. The estimated intakes would then be those shown in Table 3.

According to Table 3, the amounts of whole egg protein or equivalent would start at 4.8 gm. daily per kilo of body weight for the first nine months of life, then drop successively to 4.2, 3.6, and 2.7 gm. at 9 months, 2 years and 4 years of age, respectively. From 8 years of age through the first half of the teen-age years the level would be about 2.1 gm., then gradually drop to 1 gm. at maturity.

Support for the intake levels suggested in Table 3 for children in the 1-10 year age group is found in the investigations of Stearns and coworkers at the University of Iowa. The data reported on protein requirements (16) were based on studies of 51 children 1 to 4 years of age, and studies of 67 children 4 to 11 years of age. These workers assumed that (a) the protein requirements

TABLE 3

Estimated Minimum Daily Protein Intakes to Provide Full Labile Protein Stores

Age (years)	Protein Intake (gm./kg. B.W.)	Age (years)	Protein Intake (gm./kg. B.W.)
0	(4.8*)	12	2.1
9/12	4.2	15	2.4
2	3.6	16	2.1
4	2.7	17	1.6
6	2.2	18	1.3
8	2.1	21	1.0
10	2.1		

*Based on human milk. Other values based on whole egg protein or equivalent.

of the body are satisfied if the skeletal musculature is well maintained, and (b) the rate of growth of the skeletal musculature will be the dominating factor in determining the protein requirement of children. Growth of muscle is most rapid during the preschool years. Here weight gain is slow but type of body growth is changing and the extremities are growing more rapidly than the trunk. They recommend 3 to 3.5 gm. of protein per kg. of body weight to provide good growth of musculature in the age range of 1 to 5 years; protein allowances decreasing gradually from 3 gm./kg. but remaining above 2.5 gm./kg. are recommended for the age range of 6-10 years. They state that "a generous allowance permits better growth and maintenance of body tissue, and more adequate defenses against illness. Children so maintained exhibit abundant health."

The values presented in Table 3 are in fairly good agreement with those recommended by the National Research Council (5). They recommend 3.5 grams for infancy, 2.5-3 grams for early childhood, 1.5-2 grams in late childhood and adolescence, and approximately 1 gram for adults. However, their requirements refer to an average American diet in which the quality of the protein will be lower than that of whole egg protein.

Stuart and coworkers (17) studied two boys from infancy to 18 years of age. One was able to consume levels of calories and protein far above the recommended allowances of the National Research Council. This boy, while remaining relatively inactive, maintained low weight for height, excellent health, and a steadily high level of progress in growth. The second boy was always small and consumed during the ages of 6 to 12 years levels of protein that were low when compared with N.R.C. allowances. Progress in skeletal development was slowed during this period, there were numerous clinical indications that his health was only fair, and he manifested generally lowered resistance to infections. Johnston (18) studied growth and nitrogen balance in boys and girls just prior to and after the onset of puberty. His results led him to recommend that the optimal intake of protein for the adolescent be at least 15 per cent of an adequate caloric intake, and that the N.R.C. recommendations for protein intake of this age group be revised upward.

When less efficient sources of protein are compared with the reference protein (whole egg protein), the protein intakes listed in Table 3 must be increased. Thus, a protein with one-half the efficiency of whole egg protein should, if serving as the only protein source, be consumed at twice the intake levels shown in Table 3.

The protein quality of common foods can be expressed as the protein efficiency ratio in growing animals. In white rats this is defined as the grams of body weight gained per gram of protein consumed. Table 4 shows the protein efficiency ratio (P.E.R.) as determined in young white rats for several foods (19).

TABLE 4

Protein Quality of Common Foods*

Food	Protein Efficiency Ratio†
Whole egg	3.8
Beef	3.2
Milk	2.8
Ham	2.7
Rice	1.7
Corn	1.2
Beans, navy	1.2
White bread	1.0

*From reference 21.

†Grams gained per gram of protein consumed.

The data in Table 4 emphasize the superior quality of animal protein. This is due mainly to their better balance of essential amino acids. Cereal proteins can be improved by adding certain essential amino acids in purified form. For example, the addition of lysine at a level of 0.2% of the wheat protein to white flour will increase the P.E.R. of white bread from 1.0 to 2.0 (20) in rats. Lysine is the first limiting amino acid in wheat protein. If lysine supplemented bread also contains 3 per cent non fat milk solids, the P.E.R. is increased to 2.5, bringing it in the range of protein quality of meat and milk proteins.

Data from adult human nitrogen balance studies are now accumulating; these data show that cereal products such as bread, farina and macaroni can be improved in protein quality by the addition of lysine (21). Similar studies in young children have been reported by Scrimshaw and coworkers (22,23,24). Scrimshaw et al. (22) conclude that "by the supplementation of a vegetable protein with the appropriate essential amino acids, good nitrogen retention and satisfactory gain in weight can be obtained, at least with an intake of 3.0 gm. of protein and 100 cal. per kilogram per day in young children." The basal diet contained mainly corn masa, a product obtained by the treatment of whole corn with a heated lime solution before grinding. The essential amino acids required for maximum nitrogen retention in children were tryptophan, lysine and isoleucine, added at levels of 148, 243 and 196 mg. per gm. of dietary corn nitrogen, respectively. In their most recent experiments, lysine was found to be the only limiting amino acid in a wheat flour diet (24).

It is obvious that amino acid supplementation could serve as a powerful tool in upgrading cereal proteins, thus more readily satisfying the protein requirements based on whole egg protein as listed in Table 3. The population might then have a higher average level of labile protein stores, with greater ability to resist infection, and a greater capacity for productive work.

As part of a regional research project* on factors affecting requirements

*North Central Regional Cooperative Project NC-5. Land grant colleges cooperating.

of adult human subjects for protein and amino acids, Dr. Helen Clark of our Department of Foods and Nutrition and I have been conducting lysine requirement studies on humans. We have used a basal diet (25), the components of which are listed in Table 5. The 159 gm. of flour and 21 gm. of corn meal in

TABLE 5

Components of the Basal Diet*

Ingredient	Biscuits gm.	Corn Bread gm.	Wafers† gm.	Pudding† gm.
Flour, white wheat	138.0	21.0		
Corn meal		21.0		
Sucrose		14.0	20.0	30.0
Baking powder	5.0	1.8	1.0	
Sodium chloride	3.0	0.5	1.0	2.0
Mineral mix		0.7	1.1	
Butter oil		10.0	10.0	20.0
Vegetable fat	29.0		7.5	
Cornstarch			50.0	16.0
Mucilose flakes			2.0	
Water	85.0	43.0	30.0	200.0

*From Clark et al. (25).

†Spices or flavoring were added.

the basal diet are almost identical with the daily per capita consumption of these products in the United States (26). Baking losses of lysine were encountered in the preparation of the biscuits and corn bread (27). The basal diet provided approximately 500 mg. of lysine. The daily intake of nitrogen was maintained at 9.0 gm. Our subjects therefore had a crude protein (N x 6.25) intake of 0.86 - 1.25 gm. per kg. of body weight daily, depending on size. Equal numbers of young men and young women were used in the initial studies. In order to provide high quality protein with lysine as the only variable, purified amino acids were added to the diet to change the amino acid pattern of the cereal protein to that of whole egg protein. This is shown in Table 6. By adding the amounts of purified amino acids shown in column 6, Table 6, each subject received the quantities of essential amino acids found in 20 gm. of whole egg protein, except for lysine. The addition of 1 gm. of lysine would have been needed to equal that found in 20 gm. of whole egg protein (1500 mg.).

The final distribution of nitrogen in the diet was approximately as follows: The flour, corn meal and purified essential amino acids contributed daily 3.56, 0.28, and 0.75 gm. of nitrogen, respectively, or a total of 4.59 gm. An isonitrogenous mixture of glycine, glutamic acid and diammonium citrate contributed 4.03 gm. and fruits and other foods provided 0.38 gm.

The results of our first nitrogen balance studies with 5 men and 5 women between 23 and 29 years of age have been published (25), and show that these subjects attained nitrogen equilibrium when consuming quantities of lysine between 500 and 900 mg. No striking difference in lysine needs of men and women was apparent. To date, an additional 10 subjects have been studied. The majority have had requirements in the 500-900 mg. range.

One of the handicaps in the nitrogen balance method for measuring minimum requirements is its lack of sensitivity to changes in the labile protein stores of the body. In more recent studies (28) we encountered with one sub-

TABLE 6

Quantities and Sources of Utilizable Amino Acids Consumed Daily*

Amino Acid	Total** mg.	Flour mg.	Corn Meal mg.	Other Foods† mg.	Purified Amino Acids‡ mg.
Arginine	1280	453	36		791
Histidine	420	359	30		31
Isoleucine	1600	638	79	27	856
Leucine	1840	1216	157	53	414
Methionine	820	279	30	158	353
Phenylalanine	1260	763	52	25	420
Threonine	980	468	39	43	430
Tryptophan	300	158	6	7	129
Valine	1460	717	52	61	630
Cystine	480	204	11		265
Tyrosine	900	502	36		362

*From Clark et al. (25).

**Equivalent to quantities in 20 gm. of whole egg protein.

†As peaches, applesauce, orange and lemon juice, and a Litrison capsule that contained 150 mg. of DL-methionine.

‡L-isomers, except DL-isoleucine, of which twice the indicated quantity was used.

ject an apparent depletion of protein stores which was not evident from the nitrogen balance data (Table 7).

This subject was within the 5 per cent nitrogen balance zone (Leverton [29]) for periods 3, 4, 5 and 6 on a level of 500 mg. of lysine per day. To correct the tendency towards decreased nitrogen retention noted in period 6, an additional 455 calories were added daily in period 7. This resulted in a slight positive retention for period 7, and suggested that the 500 mg. of lysine were adequate for nitrogen equilibrium.

TABLE 7

Lysine Requirements of Subject RBC*

Period	Days	Lysine Intake (mg./day)	Caloric Intake (cal./day)	Nitrogen Retention (gm./day)
1	6	1500	3275	-0.57
2	6	1500	3275	+0.27
3	5	500	3275	-0.10
4	6	500	3275	-0.15
5	6	500	2820	-0.17
6	4	500	2820	-0.38
7	8	500	3275	+0.17
8	6	1500	3275	+1.21
9	6	1500	3275	+0.76
10	6	1500	3275	+1.26

*Male, 22 yrs., 65.8 kg. starting weight. Daily N intake: 9.0 gm.

Our complacency regarding this level of lysine for the subject was upset by his announcement toward the end of period 7 that he could not continue on the diet. He claimed that he was unable to concentrate on his studies, was tired all of the time, and feared that he would fail the mid-semester examinations which were to be given soon.

With the hope of preventing termination of the experiment, we increased the intake of lysine to 1500 mg. per day. Within 48 hours there was a marked retention of nitrogen and for a period of 18 days (periods 8, 9 and 10) the subject retained more than one gram of nitrogen daily. His mental state and feeling of well-being returned to normal and he continued on the diet squad for a total of 35 days without complaint after his daily lysine intake had been increased.

The experiences with subject RBC have been presented in detail to illustrate the complexity of balance studies in humans. The attainment of "apparent" nitrogen equilibrium for relatively long periods of time does not necessarily imply that a particular dietary regimen is adequate. During this period, a depletion of labile protein stores may be taking place, even though the loss of nitrogen from the body may be minor (see periods 3-7, Table 6, nitrogen loss in 29 days, about 3 gm.).* The depletion in labile protein reserves may have led to a reduction in levels of certain enzymes and other proteins essential to the physical and mental well-being of the human subject.

It is obvious that simple, accurate methods need to be developed for measuring the labile protein stores of the human. Work on this problem is now in progress in our laboratory.

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*Nitrogen losses in the form of perspiration and dermal growth were not measured. Although most investigators using the nitrogen balance method do not correct for these losses, they may amount to 1-1.5 gm. nitrogen per day, according to Mitchell and coworkers (30,31,32).

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II

ENERGY VALUES AND OTHER PROPERTIES OF FATS IN POULTRY NUTRITION

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More rapid growth, increased egg production and greater productive efficiency are important practical benefits which have resulted from application of principles of energy nutrition to poultry feeding in recent years. Of particular importance has been the use of inedible fats as a high energy feed resource, making possible the achievement of new highs in productive performance and efficiency.

The energy contribution of fats is their major value in animal nutrition but, due apparently to other properties of an unidentified nature, fats can produce marked improvements in metabolic efficiency and growth rate. The purpose of this paper is to review recent research on both aspects of fat in the nutrition of poultry.

ENERGY VALUES OF FATS

Much of the development of so-called high energy rations for poultry feeding has been based on empirical studies without adequate information on the energy values of feedstuffs. Further progress is partly dependent on securing accurate data on energy values of feedstuffs and purified nutrients in order to approach the problems of energy nutrition on a quantitative basis. An extensive program for determination of metabolizable energy values of various substances for poultry has been in progress in the nutrition laboratories of the Department of Poultry Husbandry at Cornell University for several years, with recent emphasis on studies of fat utilization.

Metabolizable energy is determined in our laboratories by bomb calorimetry using the chromic oxide indicator method and correcting for nitrogen retention (1). Most of the information has been obtained with young growing chicks, and in many cases confirmatory data have been obtained with adult chickens. In the case of fat, metabolizable energy is essentially synonymous with digestibility as a measure of utilization. Parallel experiments with chicks have shown that these two measures of utilization are in close agreement.

Summarized in Table 1 are metabolizable energy values of some fats and fatty acids which have been studied in this program, and representative data for a few other common feedstuffs. The fluid vegetable fats and lard were well utilized (93% or more) while pure beef tallow was more poorly utilized (67%). The feed grade tallow was composed of beef and pork fats in approximately 3:1 proportions, and its utilization was proportionately intermediate between the respective pure fats. The hydrolyzed fat was a mixture of approximately equal parts of fatty acids with neutral fats of animal and vegetable origin, and had a value similar to the feed grade tallow. Fish oils have been found to be quite high on the energy scale, approximating 87% utilization.

Differences in degree of utilization of these various fats by the chicken

TABLE 1

Metabolizable Energy of Fats, Fatty Acids and Some Common Poultry Feedstuffs

Substance	Metabolizable Energy Cal./lb.
Soybean oil	4200
Corn oil	4000
Lard (edible)	3980
Pure beef tallow	2860
Feed grade tallow	3230
Hydrolyzed fat	3230
Fish oils	3700
Stearic acid	0
Palmitic acid	0
Oleic acid	3770
Corn	1580
Wheat standard middlings	820
Soybean oil meal (50% protein)	1150

are not directly related either to degree of unsaturation (iodine number) or melting point. Of particular interest in seeking an explanation for differences in fat utilization are the data shown for utilization of stearic, palmitic, and oleic acids. The two saturated acids when fed as such are completely indigestible by the chick; in contrast, the utilization of oleic acid when fed as such is quite high. Because several of the fats showing good utilization by the chicken (such as lard) are high in palmitic acid, it is clear that its utilization in triglyceride form is quite different from its behavior when fed as a single fatty acid. Studies have also been conducted in our laboratories to determine the relative utilization of a neutral fat and the fatty acids derived from it by hydrolysis. In every case where such comparisons have been made, the fatty acid mixture has been substantially lower in degree of utilization than the intact fat. The magnitude of difference suggests that the saturated fatty acids present in such a mixture are completely unutilized (2).

Application of the data on metabolizable energy values of fat and other feedstuffs to practical nutrition of poultry for production is an important aspect of these studies. We have found with both laying hens and growing chicks that the relationship of efficiency to energy concentration of the diet is essentially linear. In other words, changing the energy concentration of the ration produces a proportionate change in the amount of ration required per unit of product (meat or eggs), indicating that metabolizable energy values are indee a valid measure of energy contribution. A change in energy concentration of 100 Calories metabolizable energy per pound in rations typical of those used in practice will produce a corresponding change of approximately 7-8% in efficiency of feed conversion (feed per unit product). The addition of 1% of fat, with appropriate adjustments to maintain nutrient balance, will produce an increase in efficiency approaching 2% (3,4). This effect is due mainly to the adjustment of feed consumption by the chicken to achieve equal energy intake from the various rations.

GROWTH STIMULATING EFFECTS OF FATS

Rigid exclusion of fat from the diet of the growing chick results in retard

ation of growth rate due, presumably, to deficiency of polyunsaturated fatty acids (essential fatty acids). Early studies by workers at Rutgers (5) and Louisiana (6) using solvent-extracted rations composed of crude feedstuffs produced growth retardation after prolonged feeding, due to an apparent fat deficiency. These findings have been confirmed with the use of highly purified fat-free diets by Bieri and coworkers (7). Significant reduction in growth rate of chicks occurred only after they had reached the age of 8 weeks. The requirement of chicks for polyunsaturated fatty acids must, therefore, be extremely small during early life, probably due in part to the carry-over of a supply in the form of egg yolk reserve.

Several reports have shown growth stimulating effects from the use of fats in simplified practical rations based largely on corn and soybean meal (8-11). These studies, as well as others in which growth stimulating effects were not apparent, have shown the expected influence of fats on efficiency of feed conversion. In recent studies at Cornell (12), marked growth stimulating effects have been obtained from the addition of fats to a purified laboratory-type diet based on glucose or starch and isolated soybean protein. Similar findings have been reported from Illinois (13) and Maryland (10), and were evident in earlier experiments at Iowa (14). In our studies maximum stimulation of growth has required a level of approximately 10% fat; increasing the level to 20% has not produced further improvement, while 5% or less has been shown to be insufficient. The most effective fats which have been studied include corn oil, soybean oil, cottonseed oil. A hydrogenated shortening was ineffective, while lard was stimulatory but evidently less effective than the liquid vegetable oils.

A similar stimulatory effect of fat has been obtained with a practical broiler ration based largely on corn and soybean oil meal, such as those typically used in broiler production. Including 7.5% soybean oil in such a ration improved growth rate by approximately 8% over a similar ration unsupplemented with fat, and reduced feed cost per pound of broiler under prevailing market prices for feed ingredients. Similar improvement was also obtained with a similar ration containing a white grease instead of soybean oil.

The explanation for the growth stimulating properties of these fats under laboratory conditions is not yet clear. There is no direct relationship between the performance of the various fats and their energy contribution, suggesting that some other property is responsible either independently of or in combination with the energy contribution. It is quite possible that an unidentified nutrient of vitamin-like nature may be involved. This is suggested by work at the U.S.D.A. Beltsville Laboratory (15), Oregon (16), and Illinois (17) which has shown marked growth stimulating effects of egg yolk which apparently cannot be explained on the basis of energy and protein contribution. Fractionation of the egg yolk has shown that the growth promoting activity is associated with the non-saponifiable portion of the fat (18).

One of the well-known properties of fat is its effect in increasing the efficiency or "economy" of energy utilization. Basic studies on this property of fat have been done at Penn State (19) and have shown that when equal intakes of energy are fed to rats from diets differing in fat content, better growth and efficiency are produced by the rations supplemented with fat. A similar action of fat in the growing chick has been shown in studies recently conducted in our laboratories (20). It is possible that such an effect may be partly responsible for the growth stimulating property of fats, but does not seem to be likely as a sole explanation.

Separation of the growth stimulating property from the energy contribution of fat is a necessary step in the study of the relationships involved. Workers at Illinois (13) have reported that feeding chicks diets containing different amounts of corn oil, with food intake limited to supply an equal amount of metabolizable energy for each treatment, the growth stimulating effect of the fat

was still evident. Using a somewhat different kind of experimental diet, contrary results have been obtained in similar experiments in our laboratories. The key to explaining the stimulatory effects of fat may be held in the differences between these experiments.

The practical implications of the new findings are not clear as yet. The preliminary observation that soybean oil is capable of stimulating growth rate in a typical broiler ration indicates that some practical benefits may be achieved. On the other hand, extensive experiments with feed grade fats at the University of Maryland (4) and elsewhere have indicated that the beneficial effects of adding low levels (2-3%) on growth rate are not improved by feeding very much higher levels of fat, even though efficiency of feed conversion is improved. If the beneficial effects of the fats in our recent experiments are indeed due to some unidentified trace nutrient, it is likely that this factor would exist in varying amounts in different fats where different lots of a given kind of fat were fed. In any case, it is quite clear that there is an effect of certain fats in improving performance of growing chicks which is not predictable from a knowledge of metabolizable energy values.

Another property of fats in the nutrition of layers also may be due to an unidentified factor or mechanism. Research workers at Washington State College (21) have observed that corn and corn oil when added to a simplified ration significantly increase egg weight.

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III

BIOCHEMISTRY OF MEAT HYDRATION

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One of the most important features of meat quality is its water holding capacity. Muscle binds its 75% water and even added water more or less tightly. Water holding capacity also plays a great role in the processing of meat. Good water holding capacity is necessary for the production of frankfurters, but for the production of salamies less water holding capacity is desired.

Higher water holding capacity results in a higher juiciness of meat after cooking. Furthermore, tenderness, color, taste and some other features of meat are related to its capacity for holding water. Also for transport, storage, canning, freezing and thawing, the water holding capacity is of great practical importance.

First of all, let me define the terms which I will use. "Water holding capacity" means the ability of meat to hold fast its own or added water during application of any force (pressing, heating, chewing, grinding and so on). "Swelling" means the spontaneous uptake of water by meat from any surrounding fluid resulting in an increase of the weight and volume of muscle. We have found that usually a good correlation exists between water holding capacity and swelling capacity of meat. The cause of both effects is the interaction between meat proteins and water. We call this interaction the "meat hydration." I know that this definition doesn't correspond exactly to the strict physical-chemical definition of "hydration" but the term is in common use and a better term has not yet been found.

A certain, sharp limit between loose and fixed bound water doesn't exist in meat. How much loose and how much tightly bound water that one finds depends on the experimental method used. Therefore, almost all methods for the determination of meat hydration give only relative figures. Nevertheless, for most purposes it is sufficient to know the relative differences of water holding capacity determined by the same method.

Mainly, we used a method (1) in which a small weighed amount of meat is pressed on filter paper between two plates. The meat is pressed to a round thin film. The area of the ring of the expressed juice, absorbed by the filter paper, is proportional to the amount of "loose" water. When I know the total moisture in meat, I can calculate the amount of bound water related to meat. The differences of the hydration of different meats become more impressive when you grind the meat sample with a certain amount of added water and press it after. There is also a certain correlation between the area of pressed meat and its hydration. Also, there is a good correlation between the water holding capacity of raw meat, determined by the press method, and the quality of the sausage (frankfurter) produced from this meat. By this simple method we can judge the meat before processing to determine if it is fit for frank-

furters (or if it is better for salamies or other purposes) (2). We have only to put a plastic film with two concentric circles on our press-paper and we can classify the meat into four different grades by this raw measurement. Sometimes, we also use centrifuge methods to determine the water holding capacity.

Now the question arises: In which way is the water bound by the muscle proteins (3)? Well, on the one hand the peptide chains of the proteins carry some free electric charges, presented by negative carboxyl-groups and the positive amino-groups (NH_3+); furthermore, they contain other so called polar groups as -OH - SH and -CO-NH- groups. On the other hand, water is a dipole; this means negative charge of oxygen and positive charge of hydrogen do not coincide and, therefore, water is a molecular magnet (Fig. 1). This magnet is attracted by all kinds of polar groups in the protein. The polar groups of the protein cause the water binding and we have some reason to assume that this electromagnetic power, this attraction, concerns not only the water bound immediately in first layer but that it also operates at some distance.

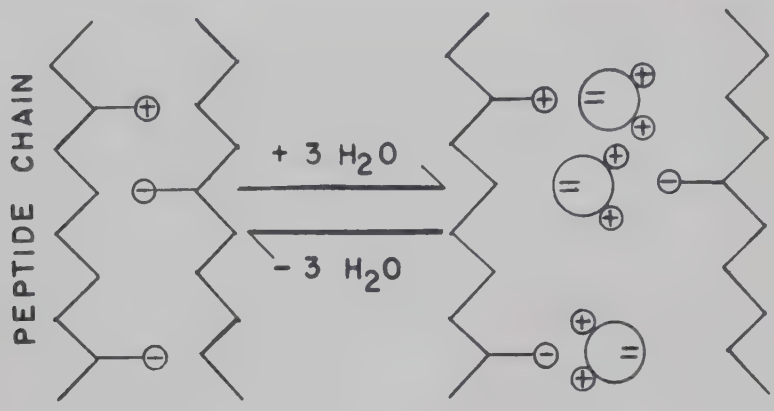

Figure 1

However, not all charged groups may bind water. Groups which compensate their charges by an inter- or intramolecular salt cross-linkage are not available for water molecules. Therefore, only the net charge of protein has an influence on the water holding capacity. I call this kind of hydration effect the "net charge effect."

But any changes of meat hydration are not due only to a change of the net charge of proteins. The peptide chains of native proteins are connected by cross linkages—for example by salt-linkages, bivalent metals, S-S-bonds, or hydrogen bonds. In this molecular network a number of charged groups are not available for the water binding because of steric reasons. There is not space enough for the water molecules. By cleavage of cross linkages the peptide chains become more flexible and now water can attach to the polar groups. I call such changes of meat hydration, which are not due to changes of net charge, the "stereo effect." Mostly the one effect will be accompanied by the other.

The influence of pH on the meat hydration is a typical example of the importance of the protein net charge (4). The isoelectric point (IP) of muscle is about pH 5.0. At this pH the amount of positive charges is equal to the amount of negative charges. In other words, at the IP the net charge of muscle protein is at a minimum. Therefore, at this pH also the meat hydration has a sharp minimum. By addition of base, the dissociation of acidic groups (negative) increases as basic groups (positive) are discharged (Fig. 2). The protein net charge increases and consequently the hydration also. The corresponding effect is caused by addition of acid. You see, the normal pH of meat—about 5.5—is close to the IP and therefore the water holding capacity is pretty low.

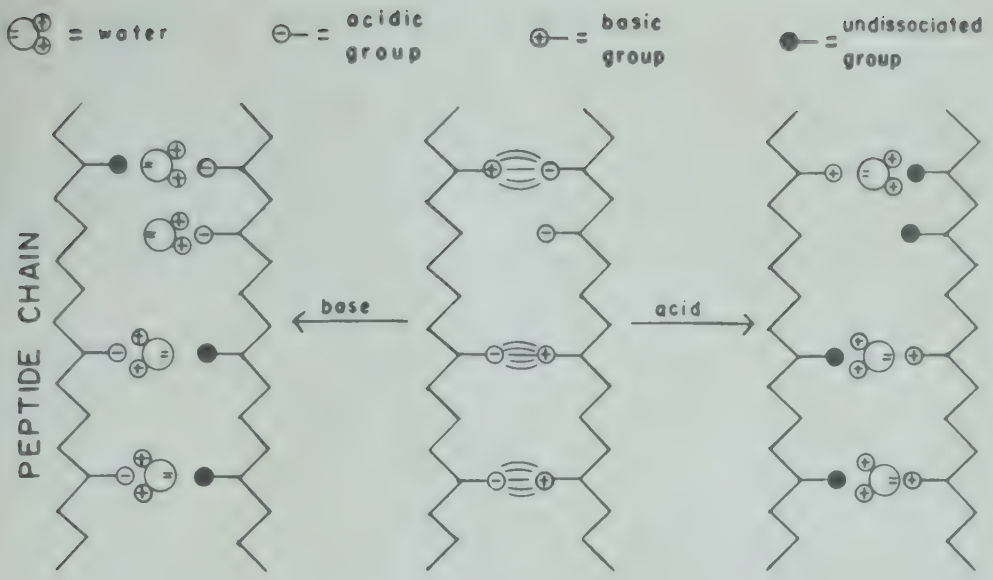

Figure 2

But any rise of pH will increase the meat hydration.

Such a pH-hydration-curve of meat (Fig. 3) gives one a good picture of the relative electrochemical conditions of your meat. One can study the influence of any process on the state of muscle by measurement of the water holding capacity at the different pH values in the range between pH 3.0 and 7.5. According to relative changes of both branches of this curve and the shift of IP one can determine if any changes of negative or positive groups or of both occurred or if there is an important steric effect. Therefore, the measurement of water holding capacity gives us not only direct information concerning meat quality but also some quite valuable information about the biochemical state of the muscle tissue. I am sorry, but I have not time enough to explain in detail the interpretation of such pH-hydration-curves, but I will tell you some of the results which we obtained by methods of this kind.

In addition to hydrogen ions for the hydration of meat, certain bivalent metallic cations are also important. These are in meat by nature and have an important influence on the water holding capacity in spite of their relatively

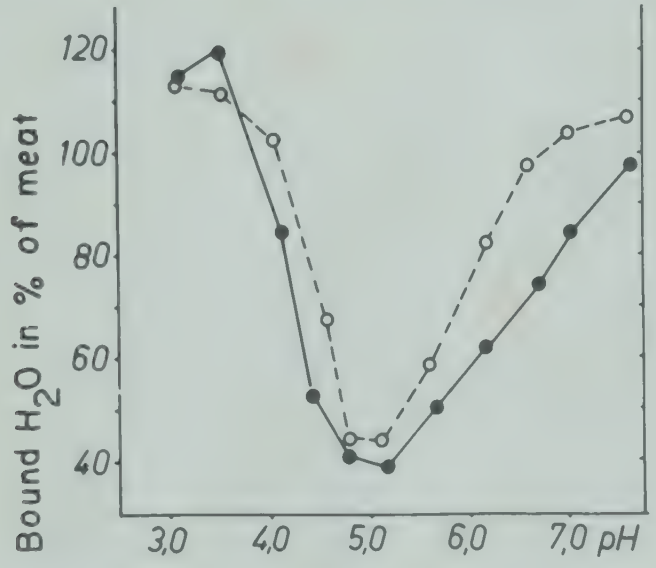

Figure 3

low concentration. We found that Magnesium (25 mg %) and Calcium (5 mg %), perhaps even Zn (3 mg %) decrease the water holding capacity of muscle (5). If we remove a part of the natural calcium from the muscle by treatment with cation exchanger (resin), then we get a strong increase of water holding capacity. This removing of calcium has an effect only at pH values higher than the IP because at the IP the cations are not tightly bound. The increase in meat hydration by removing bivalent ions is a typical case of a stereo effect (Fig. 4). By removing metallic cross linkages more charged groups become available for water binding.

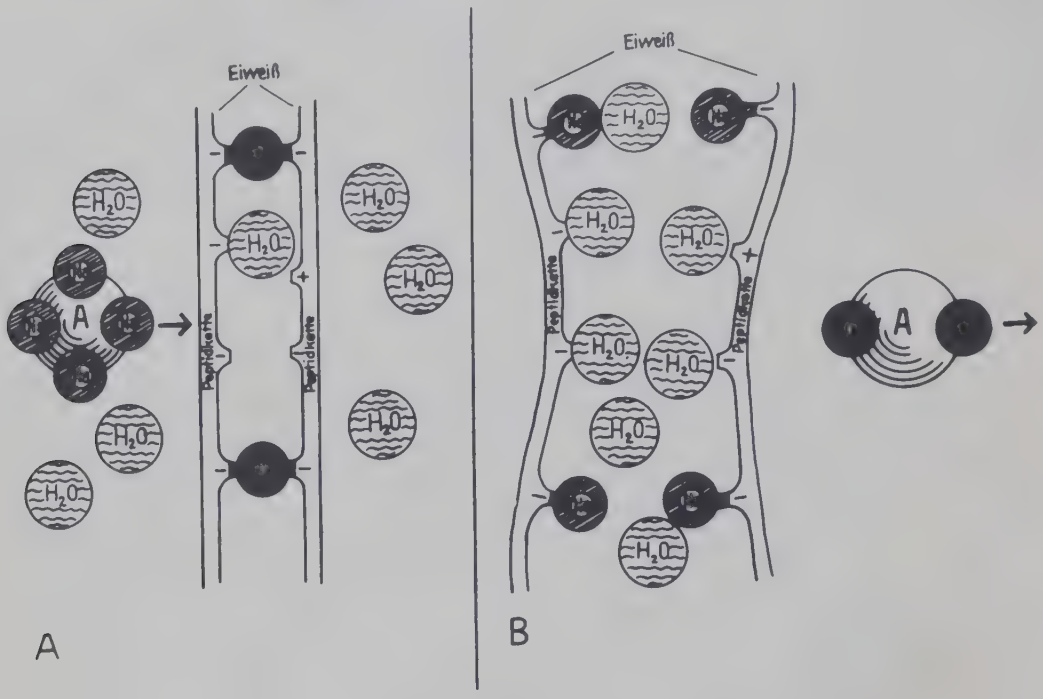

Figure 4

Changes of meat hydration after slaughtering are of great practical importance. You all know that the meat immediately after slaughtering has a pretty high water holding capacity. During the first 24 to 48 hours after slaughter, hydration decreases very markedly (6). What is the reason? Of course, the post mortem-formation of lactic acid by glycolysis results in a drop of pH and such a drop decreases the meat hydration as mentioned. But we found that only one third of the hydration-drop post mortem is explained by the decrease of pH. There must be other reasons.

Freshly slaughtered meat contains adenosinetriphosphate (ATP), an organic polyphosphate. During the first 24 hours after death this ATP is decomposed to ADP and AMP and finally, after deamination, to IMP. The post mortem drop of hydration is proportional to the decrease of ATP and we could show that two thirds of the loss of hydration are due to the loss of ATP (7). Why does ATP produce a high water holding capacity? We suppose that ATP has an effect similar to that mentioned with the cation exchanger. Because of its ability to form strong complexes with alkaline earth metals, it will tie up a part of the bivalent ions, probably the magnesium. By the breakdown of ATP its bound cations are released because AMP and IMP have a much lower ability to form complexes. Now the free cations can be built into the protein structure causing a tighter network of lower hydration (7). Actually we found that at pH 7 the structural proteins of beef muscle immediately after slaughtering contain less bound magnesium than those of the rigor or post rigor muscle (8). Corresponding to this consideration is the fact that the

change of hydration until the development of rigor concerns only the basic range of IP (Fig. 5; •—• meat, 2 hours after slaughter; o---o meat, 1 day after slaughter). During longer storage of meat the water holding capacity increases slowly. The pH increases too, but the rise of hydration is greater than corresponds to the increase of pH. Both negatively and positively charged groups increase during aging (9). We suppose that these groups become available by a steric effect—namely by the influence of proteolytic enzymes in meat. The cleavage only of few peptide bonds may cause a loosening of the network structure of meat proteins.

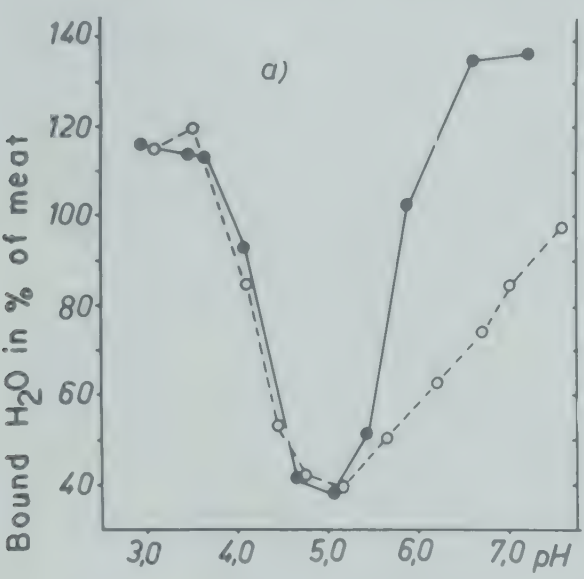

Figure 5

It is a well known fact that neutral salts have an effect on the hydration of proteins. We checked the effect of numerous anions of strong acids and cations of strong bases on the water holding capacity of meat and we found that the relative differences of the effect of all these ions on water holding capacity and swelling of muscle correspond exactly to the position of these ions in the lyothropic or Hofmeister series (10,11). In other words, these ions change the hydration of meat in the same way as the hydration of all the other proteins. We can explain the different effects of ions at different pH values in terms of charge and diameter of the hydrated ions, but I will not go too far in the theory at this time.

I shall explain only the effect of cooking salt. You know that the addition of sodium chloride increases the water holding capacity of meat pretty strongly. This effect is due to the influence of chloride ions rather than to the influence of sodium ions. Salt cross linkages between peptide chains may be split off by binding of chloride ions and there is an increase of meat hydration of both net charge effect and stereo effect (Fig. 6) (12).

As to the salts of weak acids, there are some anions which increase the hydration of muscle much more than corresponds to their position in the lyothropic series; in other words, they increase the hydration of meat more than that of other proteins. The polyphosphates and citrate, which are both used in practice, belong to this group. The effect of these salts is not only due to their relative high ionic strengths and to their influence on the meat pH. These salts work mainly by their ability to form strong complex compounds with alkaline earth metals. They eliminate bivalent cations in meat—perhaps mainly magnesium—like the organic polyphosphate ATP (13).

Now we are able to explain the effect of almost all salts on the water holding capacity of meat or to predict the effect of any salts by means of the

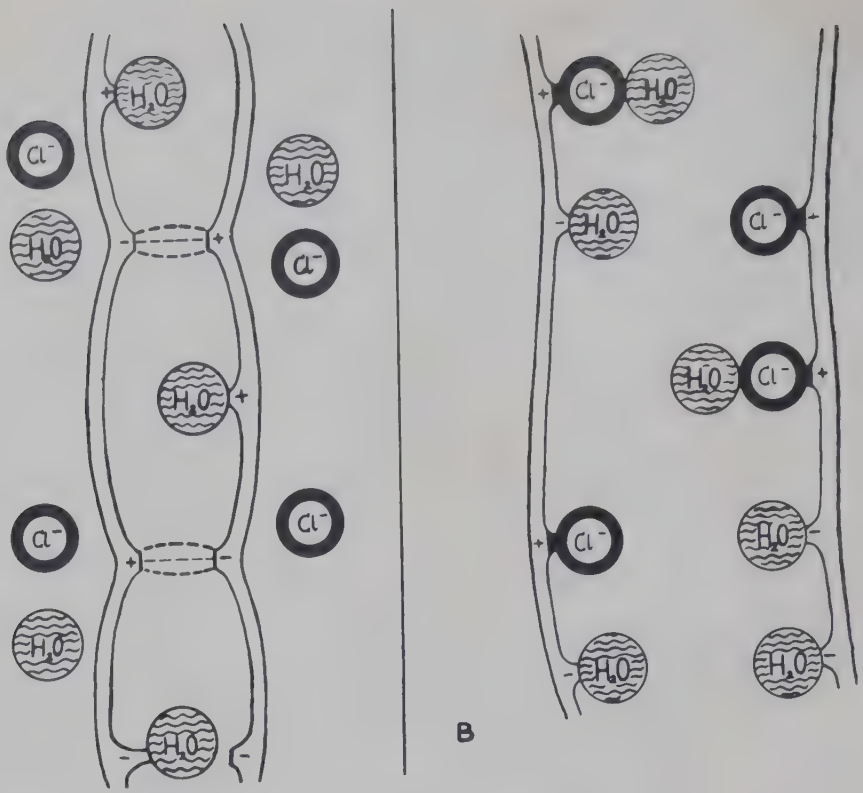

Figure 6

chemical formulas.

You can prevent the rapid loss of water holding capacity after slaughtering by salting of the ground or cut meat during the first hours after death. In this way you can preserve the high hydration of muscle at least for one week (14). I suppose that already your great grandfathers knew this fact. But we want to know the reason. ATP is broken down in the presence of salt at the same rate as without added salt. Also the association of myosin and actin to actomyosin, which is considered as the cause of rigor mortis, happens in the same way as in the unsalted meat. But no rigor occurs. The effect of presalting is as follows: Meat, salted in presence of ATP, has an extremely high waterholding capacity because of the additive effect of ATP and salt itself. Therefore, the distance between the protein chains is too great for the bivalent cations released by the breakdown of ATP to connect the chains. Therefore, we observe no drop of hydration (15). It is interesting that this favorable influence of presalting and also the influence of phosphates on the quality of sausages is due only to the hydration of the structural muscle proteins and not to a higher solubility of these proteins (15).

Grinding has an important influence on the water holding capacity of meat. Grinding increases the meat hydration because more polar groups of the proteins become available for the binding of the water dipole molecules. It makes a great difference in which manner you add the water when you grind the meat in a silent cutter. The best way is to mince the ground and salted meat without any added water by one or two rotations of the cutter. By this method the meat is cut by the knives of the cutter quite completely and therefore the now added water is bound very well. But if you add the water before grinding or during the first state of grinding, the meat fibers are distributed in the water and may give place to (make way for) the moving knives. Such meat is ground less completely, it has less surface and, therefore, the water holding capacity is less (16).

Of course, much more could be said about meat hydration. Just before I

left Germany, we found the interesting fact that the great differences in the hydration of aged meats are caused not by different pH values but by differences of the protein charges. Here in the laboratory of Dr. Deatherage we studied the influence of freeze drying, quick freezing and heat denaturation on the water holding capacity, buffer power and protein charges of beef.

I have talked only about our research in Kulmbach. But a lot of work concerning meat hydration has been done by other people—for example, by Deatherage, Wierbicki, March, Bendall, Niinivaara, Koermendy, Janicki and others.

Many problems of meat hydration are not yet solved. We know too little about the differences between different animals and between different muscles of the same animal. We know too little about the influence of breeding and feeding on meat hydration. We know nothing about the extent that different muscle proteins play in the water holding capacity of the whole muscle. We know too little about the influence of fat and the influence of the distribution of connective tissue, and so on!

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IV

DISPERSION AND RECONSTITUTION OF HIDE COLLAGEN

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Before entering into a discussion of our topic I think it best to state some of the practical reasons for the interest we at the Foundation have in this subject. Although current prices for hides are improved the substitution of synthetic materials for leather will continue. These materials can be mass produced at low unit cost and will keep pace with population trends. The recent Foreign Agriculture Service forecast (18) for reductions in hide exports suggests more immediate surpluses with consequent depression of prices. New uses, including the style trend to luxurious garment leather, leather ribbons, barbeque aprons, etc. are good medicine to offset weaknesses in markets but accumulation of hide surpluses may be expected. If the monetary value of these surpluses is to be sustained, new uses must be found which take advantage of the unique properties of the principal substituent of hides. This substance is known to chemists and biologists as collagen.

Collagen is defined by several characteristics only a few of which we will consider here. First of all, it is a protein which occurs in fibrous form. This form is responsible for the mechanical strength of the tissue it comprises and is reflected in x-ray and electron microscope patterns as well as in its resistance to the dispersing action of ordinary protein solvents. Secondly, the amino acid composition is unique as seen in Table 1. Hydroxyproline and hydroxylysine are not known with certainty to occur in any other animal protein. Glycine is the most abundant followed by proline and hydroxyproline. There are significant numbers of polar residues represented by glutamic and aspartic acids, arginine and lysine, while cystine and tryptophan are absent. Tyrosine, while present as a minor constituent, appears to bear importance in the organization of collagenous tissues. A third important characteristic is the abundance with which collagen occurs in the animal body. The estimate of 30% of animal body protein can be understood readily when one considers the values shown in Table 2. Differences with regard to collagen organization in various tissues have been amply demonstrated. Nevertheless, information gained from the study of skin applies to the understanding of connective tissues in general. That this was not always recognized is apparent from the history of collagen research. In 1900, Zacchariades (24) noted that dilute mineral acids could dissolve a portion of rat tail tendon yielding a viscous solution. Much later, from 1927 to 1937, Nageotte (12) and others (2,3) demonstrated that such material extracted with dilute organic acids when precipitated by neutralization or by addition of salt exhibited staining reactions typical of collagen. Alkaline extracts did not exhibit this property. The techniques of x-ray diffraction analysis and electron microscopy employed by Wykoff and Corey (23) and by Schmitt, Hall and Jakus (16), respectively, demonstrated still further that the precipitated material was typically collagen. The observed solubility of tendon and of fish swim bladder were considered to be a manifestation of the simplicity of organization of those particular tissues. However, in 1948 a group of Russian workers led by Orekhovitch (14,15) reported the occurrence of a collagen fraction soluble in pH 4.0 citrate buffers in the skins of several species of animals.

TABLE 1

Composition of Collagen and Citrate-soluble Collagen (1)

	g. per 100 g. Collagen	Protein Citrate-soluble Collagen
Total-N	18.60	17.70
Amino-N	0.46	0.49
Glycine	26.6	26.1
Alanine	10.3+	10.0
Leucine	3.7	3.2
Isoleucine	1.9	1.4
Valine	2.5	2.3
Phenylalanine	2.4	2.0
Tyrosine	1.0-	0.5
Tryptophan	-	-
Serine	4.3+	4.2
Threonine	2.3	2.2
Cystine	-	-
Methionine	1.0	0.8
Proline	14.4	13.0
Hydroxyproline	12.8	13.6
Arginine	8.2	8.3
Histidine	0.7	0.3
Hydroxylysine	1.2	1.3
Lysine	4.0-	3.6
Aspartic Acid	7.0+	6.0
Glutamic Acid	11.2	11.0
Amide	0.7	0.5
Hexosamine	0.05	0.01
Reducing Sugars	0.26	0.01-0.03

TABLE 2

Approximate Collagen Contents of Tissues of Various Species
Compiled from Data of Lowry, et al. (11) and of
Neuman and Logan (13)

Source	% Collagen	Reference
Chordae tendineae	76.9-84.6	(13)
Skin	64.3-79	(11), (13)
Whole bone	15.1-24.2	(13)
Aorta	23.2-31.0	(11), (13)
Liver	0.6-2.5	(11), (13)
Muscle	1.0-5.8	(11), (13)
Lung: rat	11.3	(13)
Duodenum: rat	12.0	(13)
Stomach: cardia, rat	23.6	(13)
pylorus, rat	13.0	(13)
Brain	0.22-0.36	(11), (13)

Since the yields obtainable were highest in the skins of very young animals and decreased with age it was assumed to be a precursor of mature fibers and was termed "procollagen." These observations have been confirmed by numerous investigators but considerable evidence has been accumulated to indicate that the nomenclature was prematurely applied. In 1951 Highberger, Gross and Schmitt (10) demonstrated that weakly alkaline phosphate buffers extract a lesser collagen fraction, and Harkness et al., studying the rate of incorporation of radioactive glycine into the skin of young rabbits observed that the alkali soluble fraction was in rapid equilibrium with plasma while the C-14 glycine was incorporated into the acid soluble fraction very slowly. The data indicated that the alkali soluble fraction was converted directly into insoluble collagen without passing through the acid soluble form.

More recently Gross et al. (6) made a study of the effects of pH and ionic strength upon the yields of extractable collagen with the interesting results shown in Table 3. They assume from the data that the alkali soluble fraction is also contained in the extracts obtained at neutral pH. This is perhaps a valid assumption in view of the fact that Orekhovitch observed high turnover rates when studying the uptake of C-14 labeled glycine into acid extractable collagen. With regard to ion concentration it is particularly interesting to note that ionic strength 0.45 is the more efficient for both phosphate and citrate buffers. These investigators postulate that th neutral salt soluble fraction exists as a prefibrous tropocollagen, while the status of the acid soluble "procollagen" is uncertain.

TABLE 3

Collagen Content of Salt Extracts of Calf Corium (6)

Extractant	Ionic Strength	pH	Collagen (Mg./Ml. in Extract)
Phosphate	0.45	8.0	1.1
Phosphate	0.45	7.6	0.74
Phosphate	0.45	7.0	2.7
Phosphate	0.19	5.9	0.76
Citrate	0.45	4.0	5.3
Citrate	0.19	4.0	3.8
NaCl	2.57	Unbuffered	0.34
NaCl	1.7	Unbuffered	0.8
NaCl	1.5	Unbuffered	0.68
NaCl	1.0	Unbuffered	1.5
NaCl	0.7	Unbuffered	0.89
NaCl	0.45	Unbuffered	0.55
NaCl	0.2	Unbuffered	0.23

The reconstitution of soluble collagen has been the subject of several investigations and details can be of considerable value both in the study of experimental material, and perhaps later in quantity production. In some of the early work with the electron microscope, tissue slices were shadowed with chromium vapors and the connective tissue exhibited a mass of fibers with regular cross banding as seen in Figure 1. The distance between these striations averages 640 Angstrom units and this spacing is characteristic of the so-called native fibril. The form of the reconstituted fibril has been shown in numerous instances with collagens obtained from tendon and fish swim bladder as well as skin to depend upon salt concentration, pH, and in some instances, the presence of certain organic substances (5,10). Dilute acetic acid extracts filtered through fine porosity filters show only fine, structureless filaments,

and when dialyzed against water form gels which fail to exhibit typical collagen fibrils. When NaCl solutions of molarity ranging from 0.003 to 0.2 are substituted for water in the dialysis, fibrils of the typical native spacing of 640 Angstroms are obtained. When the salt concentration is raised to 0.3 to 0.3 M the spacings become much more compact; the regular periods are only 220 Angstroms apart or only about one-third that found in native fibrils. At still higher salt concentrations the fibril banding disappears altogether. Fitton Jackson and Randall (4) observed with a solution of tendon collagen that normal fibril formation was favored in the region of the isoelectric point at pH 5.8. Typical electron microscope patterns were obtained by direct precipitation of the fibrils onto the specimen holder in the pH range of 4.8 to 7.2. This was accomplished by addition of calculated amounts of dry sodium acetate to acid solutions of the collagen and demonstrates the rapidity with which the fibrils can form under favorable conditions.

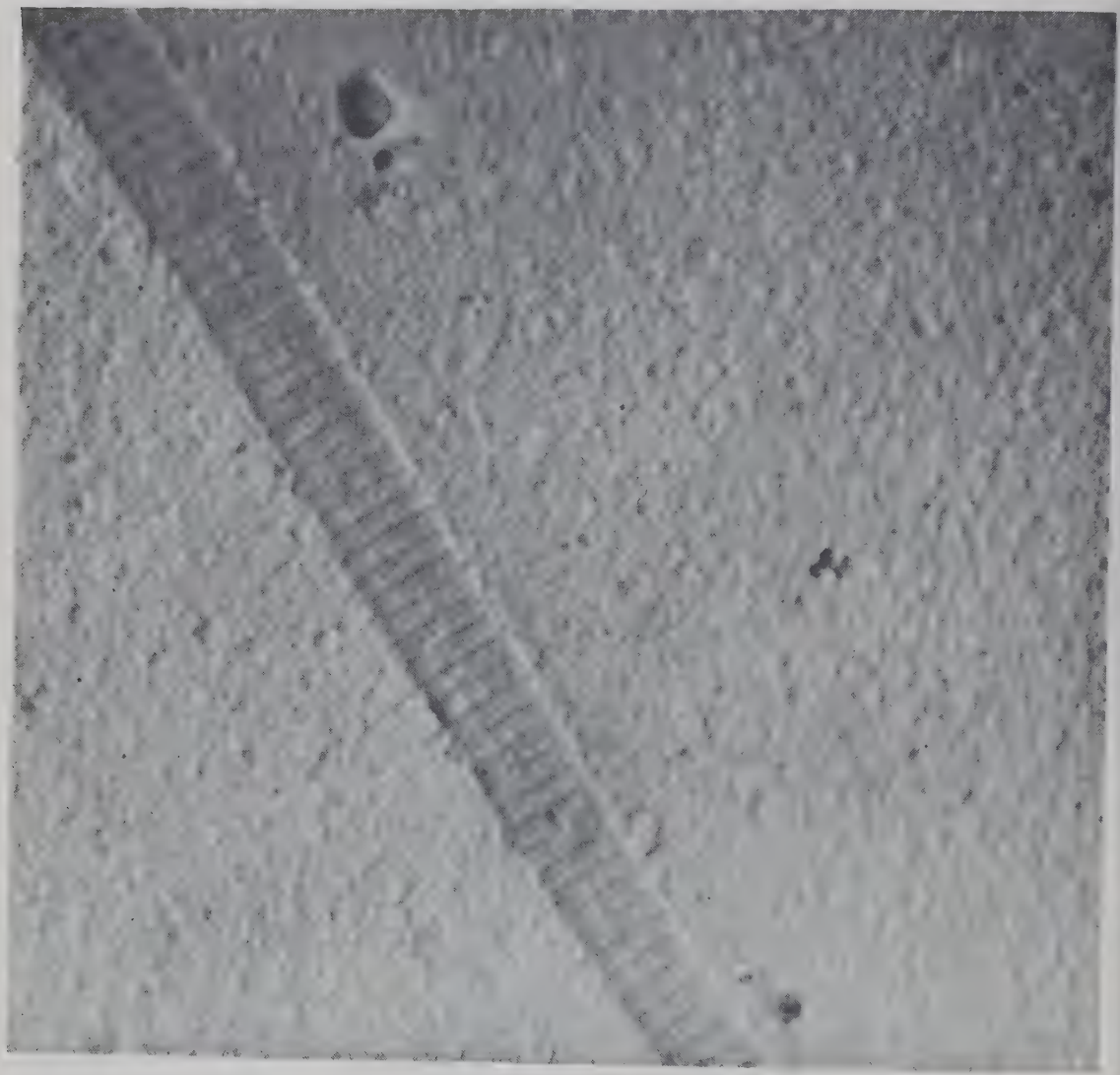

Figure 1. Reconstituted collagen. Native type spacing.

A different fibrous form was first observed by Highberger, Gross and Schmitt (9) when they dialyzed acid soluble "procollagen" type preparations from skin against water. They discovered that this new type of fibrous long spacing, termed FLS, required an inducing substance. Prolonged extraction of the skin with pH 9 phosphate prior to acid extraction removed this substance,

and these same alkaline extracts upon dialysis against water, solution in citrate buffer and redialysis yielded FLS fibrils. A number of negatively charged, high molecular weight substances such as plasma alpha-glycoprotein, chondroitin sulfate, and heparin produce this form in collagens from a variety of sources. All of the reports in the literature wherein FLS is obtained indicate a dialysis procedure necessary. Still another form which is somewhat of a curiosity is the so-called SLS or segment long spacing form. It can be precipitated directly from phosphate solutions by addition of ATP. A number of other relatively non-specific inducing substances are active when added to the phosphate solutions prior to the dialysis against citrate. They include such substances as chondroitin sulfate and nucleic acids. In contrast to the native space of 640 A° the FLS spacing ranges from 2000 A° to 3000 A° while the SLS spacing is also variable between 1600 A° and 2400 A°.

Of greater value from a practical point of view is the observation of Gross et al. (6) that neutral salt soluble collagen set to gels when heated to 37° C. These gels become increasingly stable with increased holding times and are resistant to the action of extractants used to obtain them. This is in contrast to the behavior of collagen precipitated by dialyzing away the solubilizing salts. Both gels and dialyzed precipitates exhibited typical collagen fibrils under the electron microscope.

Although some connective tissues such as tendons can be largely dispersed by modification of the techniques just discussed the combined yields obtainable from mature hides would be on the order of 10 per cent of the total. The extractions just described are performed at temperatures slightly above freezing, and the fractions obtained at these temperatures, i.e., the acid soluble, the neutral salt- or alkali-soluble, and insoluble residue are often referred to as the three forms of collagen. The "insoluble" residue has been the target of Veis and Cohen in a series of elegant reports (19,20,21,22) published in recent years. They studied the action of acid at 60° C. in the pH range 5.7 to 2.0. At pH 4 and above about 15% of the tissue was dispersed in 4 hours; at pH 3 the fraction increased to about 25%; pH 2.5 yielded 40% while at pH 2.0 about 75% dissolved. At the end of 4 hours at pH 2 more rapid degradation was setting in but at each of the less acid extractions a discrete plateau of solubilized material was seen. Ninety-five to 100 per cent of the solubilized material was retained by dialysis membranes. A range in molecular weights from 1×10^6 to 20×10^6 suggests that the dispersed aggregates may contain appreciable quantities of intact tropocollagen of molecular weight 340,000. These investigators are reporting upon the persistence of collagen structure in the coming American Chemical Society meetings.

Another method of dispersing hide collagen was investigated by Thomas in 1925 (17) while he was seeking a preservative superior to NaCl. His investigations with a number of halides included $CaCl_2$, $MgCl_2$, NaBr and others and were performed at room temperature. He noted that in one week up to 20 per cent of the total nitrogen was solubilized by some 2 Molar salts and very prolonged treatment dispersed up to 60% of the hide. He termed the observed effect hydrolysis but was criticized by Gustavson (7) who thought molecular compound formation with the salts a more plausible interpretation.

In our studies at the Foundation the use of $CaCl_2$ solutions was initially prompted by the fact that 10 per cent solutions of the salt are known to extract chondroitin sulfate and some of its collagen complexes. Since chondroitin sulfate removal has increased the solubility of some tissue collagens the $CaCl_2$ reagent was used on the center corium slices. Following cold room extraction of the tissue with 10 per cent sodium chloride, pH 9.0 phosphate, and pH 3.7 acetate buffer, the $CaCl_2$ was seen to have a specific effect in markedly reducing the tyrosine content of the tissue residue. Upon exhaustive dialysis against tap water, then distilled water, white, opaque gels were obtained which became increasingly firm with time. The gels are readily com-

pressed to a fibrous mass by gentle pressure in much the same manner as a sponge, but without observable elasticity. Paper chromatography of acid hydrolysates of acetone dried fibers yielded an amino acid pattern typical of collagen. Electron microscopy revealed that initial extracts were composed of normally banded fibrils (Fig. 1) while later extracts exhibited only fibrous long spacing (Fig. 2). These observations indicate the persistence of an inducing substance through prolonged and repeated extractions.

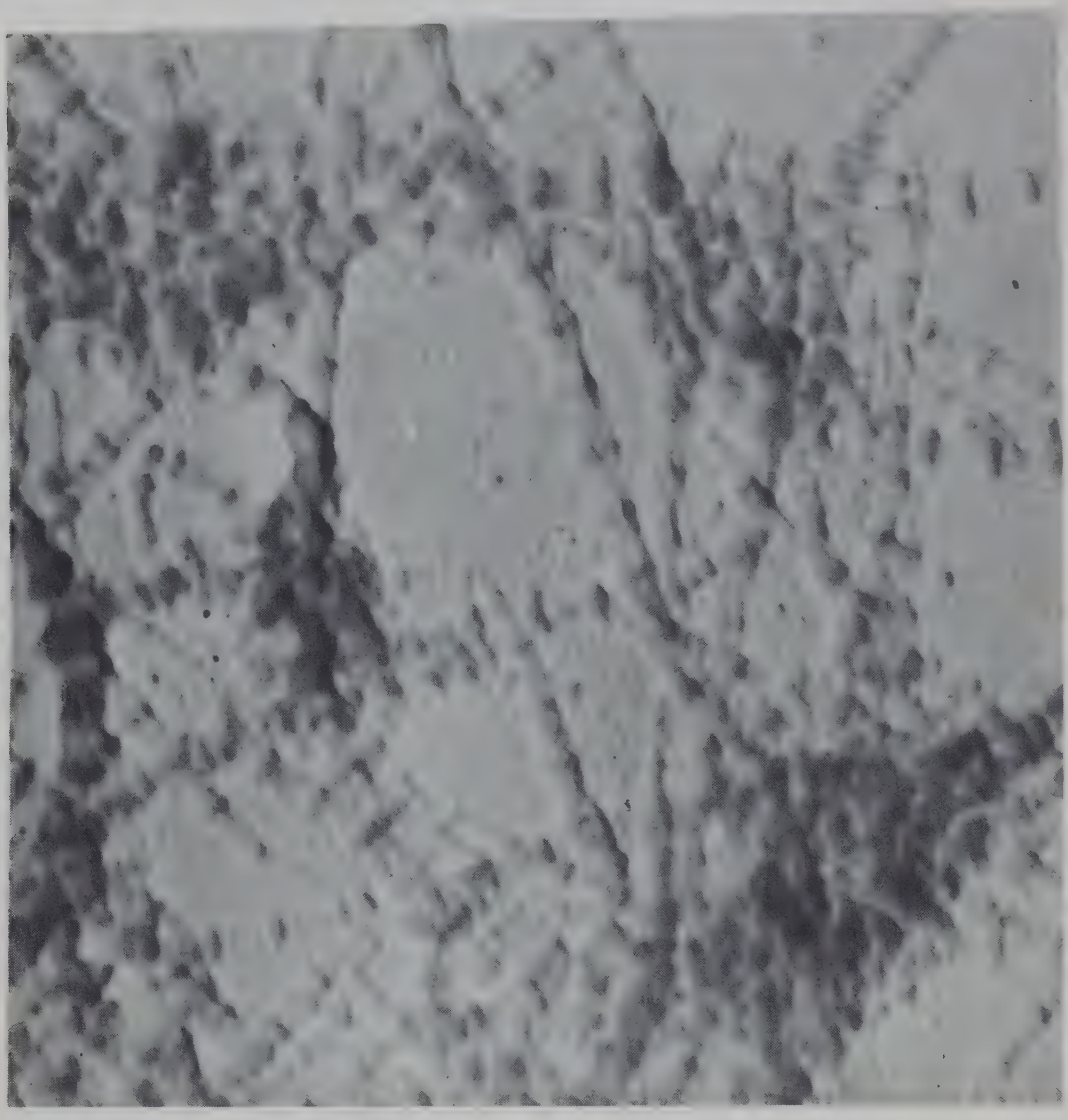

Figure 2. Reconstituted collagen. Fibrous long spacing.

This preliminary sketch has been confined to the study of ten per cent $CaCl_2$ extracts. It remains to be seen whether the large yields of extractable collagen obtained with higher concentrations of $CaCl_2$ and other lyotropic agents can be manipulated to obtain roughly equivalent yields of reconstituted collagen. It would be interesting to observe the effects of such reagents upon the high molecular weight fractions obtained using acid at moderate temperatures.

In conclusion, it appears quite possible that a combination of techniques could yield quantities of reconstituted collagen sufficient to explore and supply new uses for the surpluses of the industry.

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V

HIGH TEMPERATURE AGING OF BEEF*

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In a period when food manufacturers have produced numerous new convenience products to compete for the consumer's dollar, it becomes particularly important that the appealing qualities of fresh meats be maintained and, if possible, improved. Eating satisfaction in beef is dependent upon several characteristics, but tenderness is of greatest significance.

The tenderness of beef may be increased by aging or holding carcasses or wholesale cuts at refrigerated temperatures, but this procedure normally is reserved for the hotel and restaurant trade and other specialized markets. The development of an economic procedure to rapidly age meat would not only improve the tenderness and acceptability of the meat which is usually found in our retail stores, but also would result in a greater acceptance of meats from lower grade carcasses. Proteolytic enzyme preparations from vegetable sources are being used to improve the tenderness of beef cuts which are frozen, or are cooked immediately after treatment. A second approach to the rapid tenderization of beef is to accelerate the action of the native enzymes which are assumed to be responsible for tenderization in normal aging practices. This method is embodied in the Tenderay process of the Kroger Company in which previously chilled meats are exposed to ultraviolet irradiation at 67° F. for approximately two days (2). With the aid of antibiotic infusion or ultraviolet irradiation beef rounds and carcasses have also been aged in the temperature range of 65° to 86° F. (4,5). Experimentation at temperatures in excess of this range has been limited because of the anticipated spoilage problems.

In the work here reported both antibiotics and gamma irradiation were used, when necessary, to permit an investigation of aging temperatures of 90° F. and above with the thought of determining the optimum aging conditions for the rapid tenderization of beef. A more detailed report of this work has been prepared for publication elsewhere.

For most of the experiments the beef was aged as steaks in order that adjacent muscle locations could serve as control and treated lots. Previous work in this laboratory (1) had characterized the tenderness pattern in the semimembranosus. On the basis of this work, steaks numbered four through nine, as removed from the anterior end of the semimembranosus, were selected as being the most suitable for this study. These steaks were randomly assigned to the experimental designs so that, as far as possible, all animals, sides and locations within the muscle were represented at each time and temperature of aging.

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*This is a report of work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by Mr. William L. Sulzbacher of the Eastern Utilization Research and Development Division of the Agricultural Research Service.

Chilled inside rounds of U. S. Good and Utility quality were obtained from a commercial slaughterer 48 to 72 hours after slaughter. When antibiotics were used as a preservative, a solution was stitch pumped into the top round prior to removing the steaks. Irradiation was carried out after the steaks were packaged. Refrigeration sufficient to maintain a steak temperature below 40° F. was provided by interleafing refrigeration plates (Can-pro) between the steaks. In exploratory experiments it was determined that a calculated level of 20 to 50 p.p.m. of oxytetracycline was required to give sufficient distribution of the antibiotic by the stitch method. It was assumed that 10 per cent of these levels would have been sufficient if uniform distribution were achieved. In combination with this antibiotic level, 45,500 rads of gamma irradiation were found sufficient to control bacterial spoilage under the most severe aging conditions. This low level of irradiation also avoided the development of radiation induced, undesirable flavors which were noted when levels of 100,000 rads or more were employed.

The effectiveness and safety of the preservative methods were determined by 1) differential plating for aerobic and anaerobic bacteria, and for toxigenic staphylococci, 2) gram stained impression smears, and 3) intraperitoneal injection of mice with the supernatant from the meat and water homogenate used for bacteriological platings. Control and experimental samples were frozen until the bacteriological examination was completed. The steaks were defrosted in the refrigerator and broiled by standard cooking procedures to an internal temperature of 150° F. A panel of eight members scored the steaks for tenderness and were asked to comment regarding the flavor and texture of the samples.

Following the procedures outlined, eight pairs of U. S. Good rounds were infused with 30 to 50 p.p.m. oxytetracycline and sliced into steaks. After vacuum packaging in CRY-O-VAC they received 45,500 rads of gamma irradiation and were aged for 0, 1, 2, or 3 days at 90° F., or held at 35° F. as controls. The higher level of antibiotic was employed to provide more adequate microbial control in those steaks which were held for three days.

The scores for initial tenderness of steaks aged at the two temperatures and the differences between the means are shown in Figure 1. The tenderness

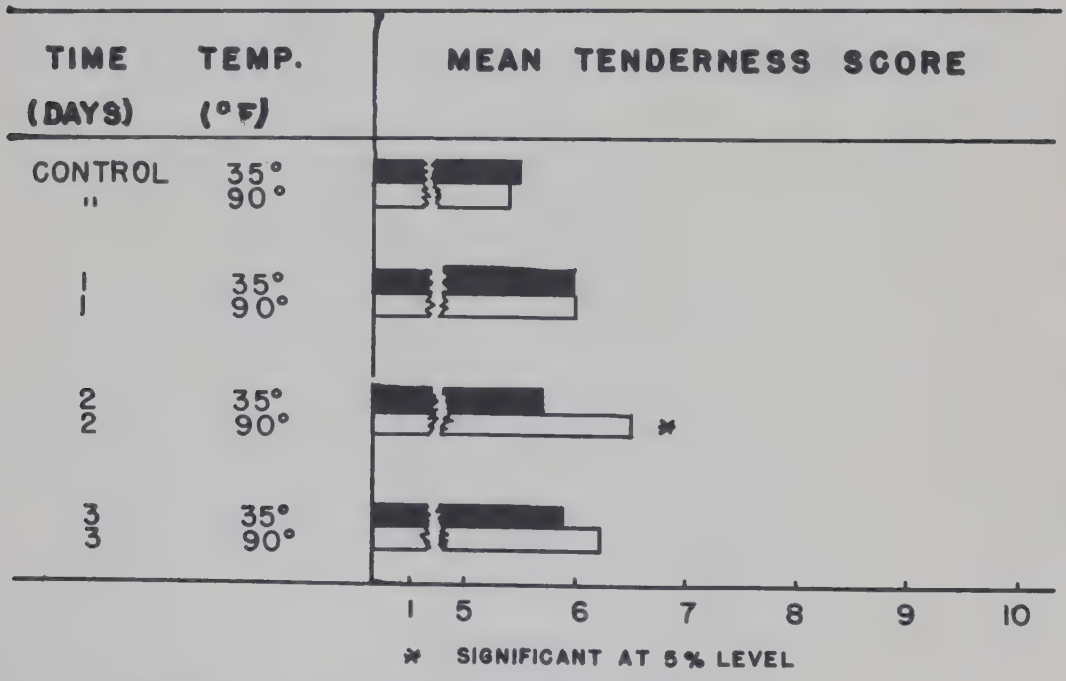

Figure 1. Mean tenderness scores of steaks aged at 35° F. and 90° F. for 1 and 3 days.

of the steaks held at 90° F. increased over the control group at each holding time with the greatest difference occurring after 2 days aging. The mean tenderness score for the control steaks also increased during holding but not to the degree observed in those held at 90° F. The steaks aged two days at 90° F. were significantly more tender than the most tender group of steaks held at 35° F. These results suggested that two days was sufficient to obtain tenderization in steaks held at 90° F.

In some preliminary experiments it was determined that antibiotic treatment alone was sufficient to control bacterial growth in steaks held at 110° F. for 48 hours. This was generally confirmed in the second series of experiments in which steaks from four pairs each of Utility and Good grade rounds were aged at 110° F. for a maximum of 40 hours. Following the infusion with oxytetracycline, the steaks were allocated to either a 35° F. or 110° F. aging temperature for 0, 16, 24, or 40 hours. A summary of the mean tenderness scores of the combined data for both grades, and the differences between them is shown in Figure 2. Tenderness scores for steaks from U. S. Good carcasses were scored approximately one unit higher on the tenderness scales, but the effects of aging were similar in both grades. Within each the increase in tenderness was greater in the steaks held at 110° F. than in those held at 35° F. When the data from both carcass grades were pooled, the largest increase in tenderness was produced by aging for 24 hours at 110° F.

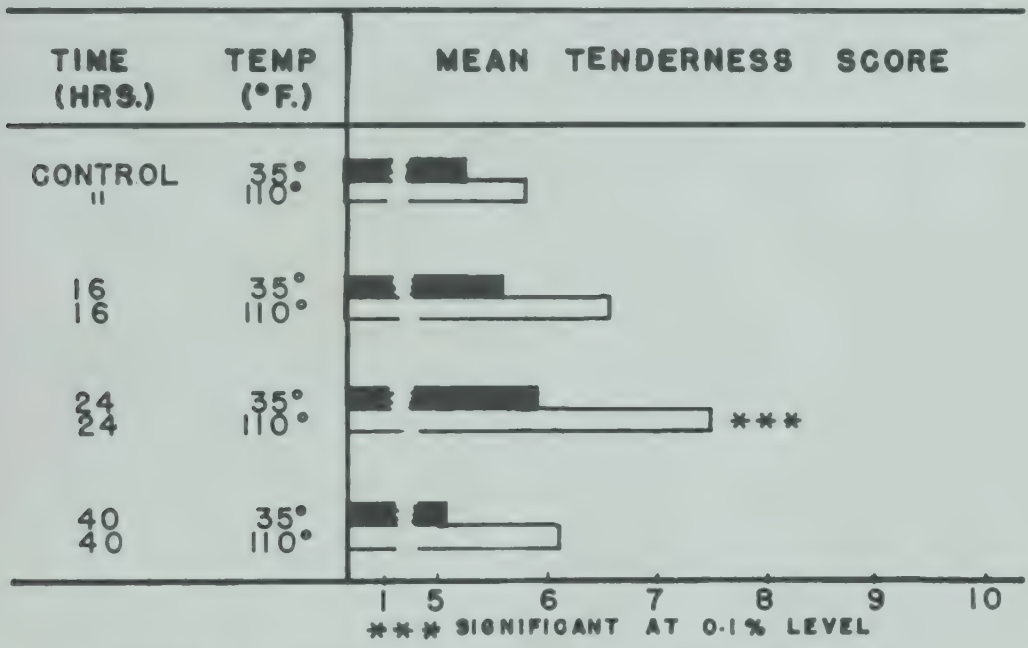

Figure 2. Tenderness scores of Utility and Good grade steaks aged at 35° and 110° F.

A further experiment was designed to compare steaks aged for 48 hours at 100° F., 24 hours at 110° F., and 24 hours at 120° F. Six pairs of rounds of U. S. Good quality were stitch pumped with 50 p.p.m. oxytetracycline. The steaks which were aged at 110° F. received approximately 45.5 thousand rads of gamma irradiation prior to aging. A comparison of the average taste panel scores for each of the treatments is shown in Figure 3. Each of the aging treatments at high temperatures significantly increased the tenderness over the unaged control sample. The most improved tenderness occurred in those steaks aged at 120° F. for 24 hours.

In the lower half of Figure 3 a comparison is made between the three high temperature aging conditions and shows that 120° F. had the most pronounced effect. Forty-eight hours at 100° F. produced the same tenderization

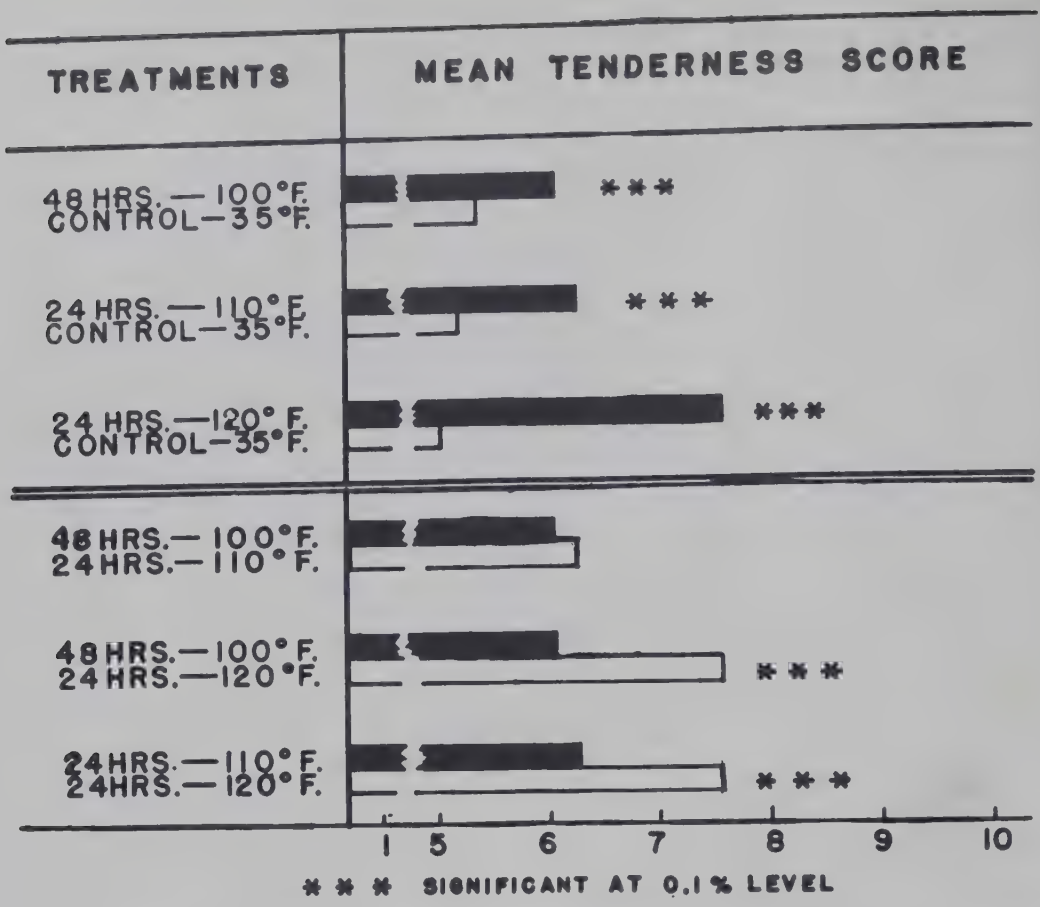
TREATMENTS
MEAN TENDERNESS SCORE
48 HRS. — 100° F.
CONTROL — 35° F.
24 HRS. — 110° F.
CONTROL — 35° F.
24 HRS. — 120° F.
CONTROL — 35° F.
48 HRS. — 100° F.
24 HRS. — 110° F.
48 HRS. — 100° F.
24 HRS. — 120° F.
24 HRS. — 110° F.
24 HRS. — 120° F.
* * *
1 5 6 7 8 9 10
* * * SIGNIFICANT AT 0.1 % LEVEL

Figure 3. Tenderness scores of steaks aged at 100°, 110°, and 120° F.

as 24 hours at 110° F. The apparent advantage of aging at 120° F. was rather misleading because members of the taste panel indicated that the steaks had an old or "warmed over" flavor and odor. It was also noted that the pigment of the aged steaks prior to cooking was rather severely oxidized. These observations implied that the meat was being cooked (collagen hydrolyzed) at this temperature; and therefore, the treatment was eliminated from further tests.

In the foregoing experiments the rapid aging procedures were compared with adjacent steaks held at 35° F. for the same time period. A further experiment was conducted to determine whether the increases in tenderness at the high temperatures were comparable to those produced by aging steaks two weeks at 35° F. Using procedures similar to those outlined above it was found that steaks aged at 35° F. increased in average tenderness score from 5.2 to 5.9, while those aged at 110° F. increased from 5.3 to 6.3. A statistical analysis of these data indicated that the two methods were comparable with respect to the increased tenderness produced.

Application of the laboratory experiments to carcass aging was evaluated on a limited scale. Preservation of the carcasses during aging was provided by oxytetracycline injected two hours prior to slaughter. An intraperitoneal injection was used for the first steer and an injection in the distal portion of the tail was employed in the remaining four. Steers weighing 950 to 1000 pounds, and carrying sufficient finish for Good grade carcasses, were chosen and given from 4 to 8 grams of oxytetracycline in a citric acid solution by one of the two procedures. Immediately following slaughter the carcasses were brought to the laboratory, and the right hind quarter and rib were placed in an air-conditioned chamber at 120° F. and maintained at 80-90 per cent relative

humidity. This temperature was maintained until the internal temperature of the rib was raised to 110° F. The temperature of the storage chamber was then reduced to 110° F. for the remainder of the 24 hour aging period. It was found that the relative humidity could be maintained at 90% without moisture condensation on the carcass, and that no treatment was required to control external microbial growth. Under conditions of high relative humidity carcass shrinkage was held to 2.5 to 3.0 per cent over the 48 hour aging and chilling period.

The left side of each carcass was held at 35° F. for two weeks as a control. A measure of the tenderization which occurred at 110° F. was made by comparing a sample removed from the chilled sides three days after slaughter with a similarly located sample from the sides aged at high temperatures.

An assay of the loin rib eye indicated that a level of .5 to 1.5 p.p.m. of oxytetracycline was present in the carcasses following aging.*

Microbiological control during aging was satisfactory in four of the five carcasses. Cultures of muscle samples from the second carcass which was aged at 110° F. showed evidence of a moderately high population of anaerobic, gas-producing organisms. Subsequent work on the remaining carcasses suggested that the sample of the second carcass may have been contaminated during cutting and sampling procedures.

Following aging, steaks from comparable left and right side locations in the longissimus dorsi of the rib, and the biceps femoris, semitendinosus and semimembranosus of the round were removed for taste panel evaluation. The mean tenderness scores for each of the muscles aged by the two methods are shown in Figure 4. The taste panel scores for tenderness of the unaged control steaks and those aged at 110° F. are also indicated. Aging at 110° F. increased the average tenderness score of the longissimus dorsi from 4.7 to 7.6. This difference was considerably greater than had been observed with steaks aged at similar temperatures and may be related to the reduced time between slaughter and freezing the steaks. Except for the semitendinosus, there was no significant difference between the tenderness scores of steaks from the sides aged at 110° F. for 24 hours and those aged at 35° F. for 2 weeks. In this instance the steaks from the sides aged at 35° F. were more tender than those aged at 110° F. The average score for all steaks was slightly higher for those aged at 35° F. but the difference of 0.2 units was not statistically significant.

Other changes occurred in the carcasses aged at 110° F. Some of the muscles in the carcasses were considerably lighter in color. This was not consistent for the same muscle in all carcasses but seemed to occur most frequently in the longissimus dorsi. Upon exposure to air, the light colored muscles "bloomed" normally but had the appearance of beef from younger animals. Some undesirable flavors were noted in the steaks from sides aged at 110° F. These were similar to those noted in the steaks aged at 120° F. but were much less pronounced. Further evidence of the flavor changes was noted in the differences between the TBA values for meat aged under the two temperature conditions. Further study is required to determine the nature of the undesirable flavors and to determine if they could be further reduced by the selection of more ideal aging conditions.

These studies have shown that effective tenderization occurs in carcasses aged at 110° F. The increase in tenderness brought about by holding carcasses at this temperature for 24 hours are comparable to those obtained in two weeks at 35° F. Commercial application of this method of aging will depend upon some further refinement to control flavor changes, the acceptance of low levels of antibiotics as an aid in holding beef carcasses and the development of facilities to process carcasses at elevated temperatures.

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*Assay performed by Chas. Pfizer & Co., Inc.

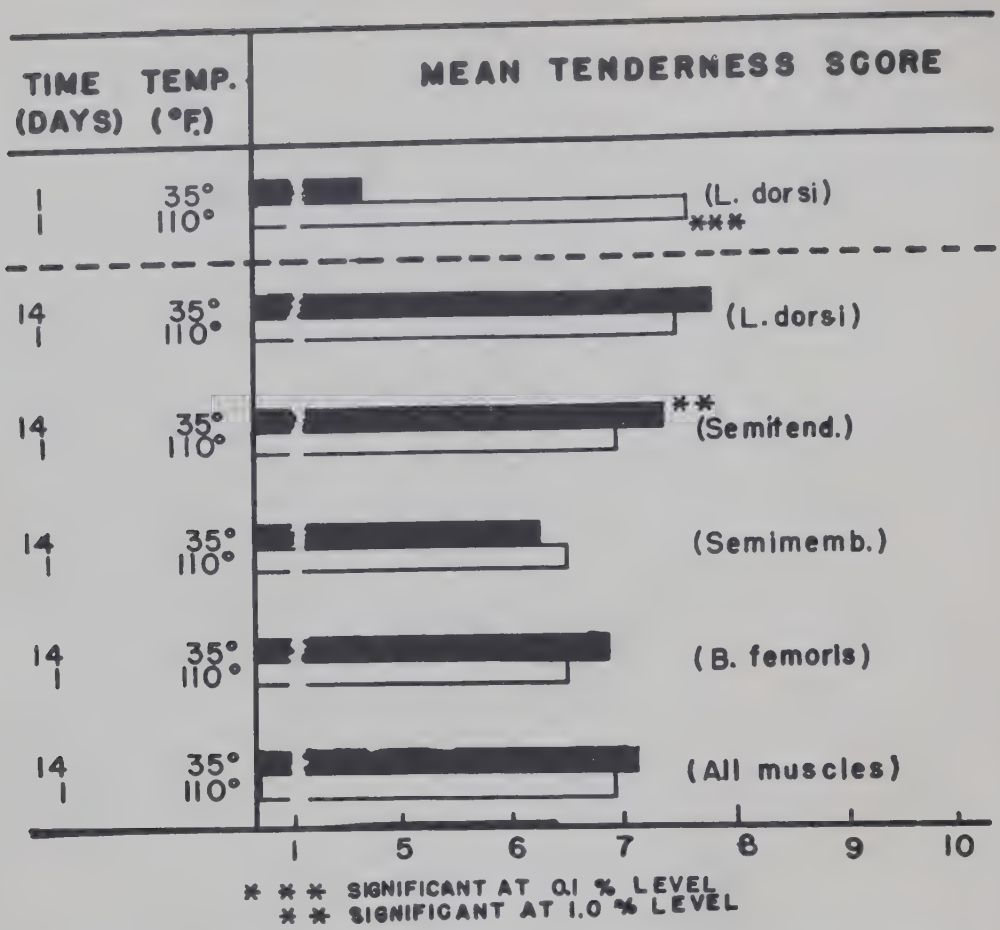

Figure 4. Tenderness scores of steaks from carcasses aged at 35° and 110° F.

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VI

THE FREEZING OF TISSUES AS SEEN AT THE MOLECULAR LEVEL

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To bring into full light the mechanism of freezing of biological material one must picture it at the molecular level; more specifically, one must visualize the process of formation, development and growth of the ice crystals from water molecules as the building blocks, and the process of withdrawal of these molecules from their substrate. To paint that picture has been my aim in a long-range research program, in which the formation of ice was to be studied, first, in purely physical systems (solutions of crystalloids and of colloids), and the knowledge thus acquired was, then, to be applied to cells and tissues. A first stage of the work on physical systems has been completed and we began the study of the freezing of tissues. Our first attempt has been with muscle, and this report will concern only that tissue.

My analysis of the process of freezing will be divided into four sections: I. A short survey of the principles of construction of ice crystals from water molecules; II. A summary of our essential findings on ice formations in aqueous solutions; III. A comparison of ice formations in such solutions and in the fluids of the muscle (a structureless medium); IV. A study of the mechanism of freezing in muscle fibers (systems possessing a structure).

I. PRINCIPLES OF CONSTRUCTION OF ICE CRYSTALS

The molecules of water, H-O-H, are understood to be of the general shape represented in Figure 1. Each of the two atoms of hydrogen behaves as if it were carrying a positive charge (sign + in the figure) and the atom of oxygen, as if carrying two negative charges (sign -). Thus each molecule can become attached to another at any of four points.

Some of the simplest structures formed by this mode of attachment are rings consisting of six molecules. (Three such rings are shown in Figure 2, in which the molecules of water are represented by spheres.) These hexagonal rings are connected into reticular layers (Fig. 2). The layers are super-

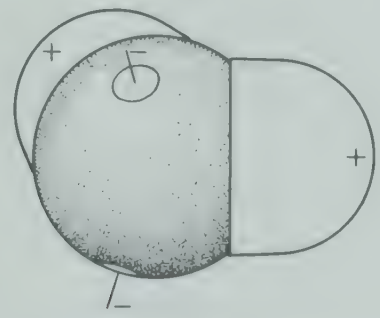

Figure 1. Model of the molecule of water.

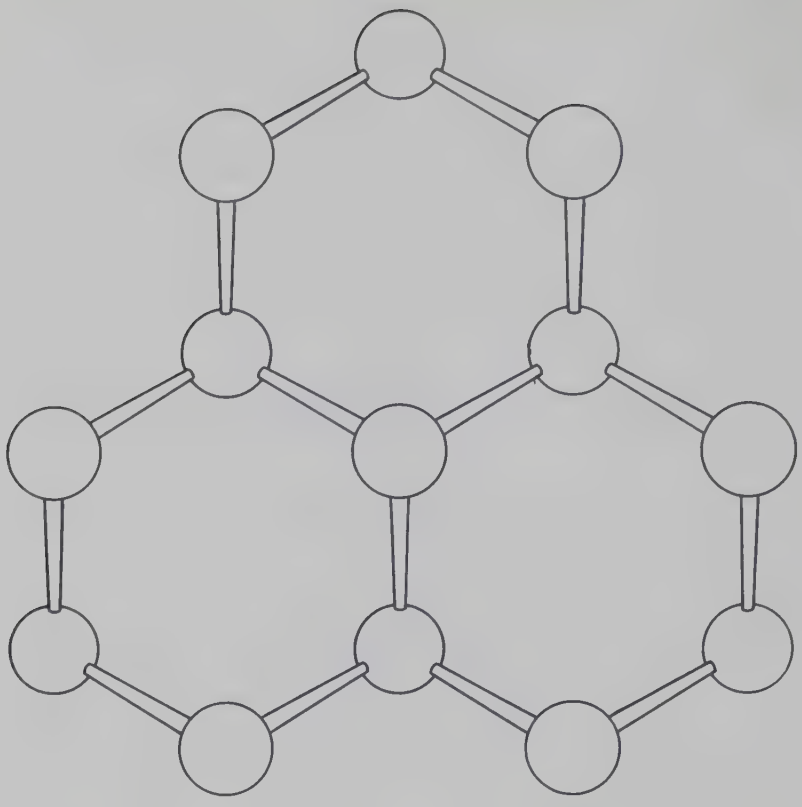

Figure 2. Arrangement of six molecules of water into a hexagonal figure, and of these hexagonal figures into a "basal plane" in the ice crystal.

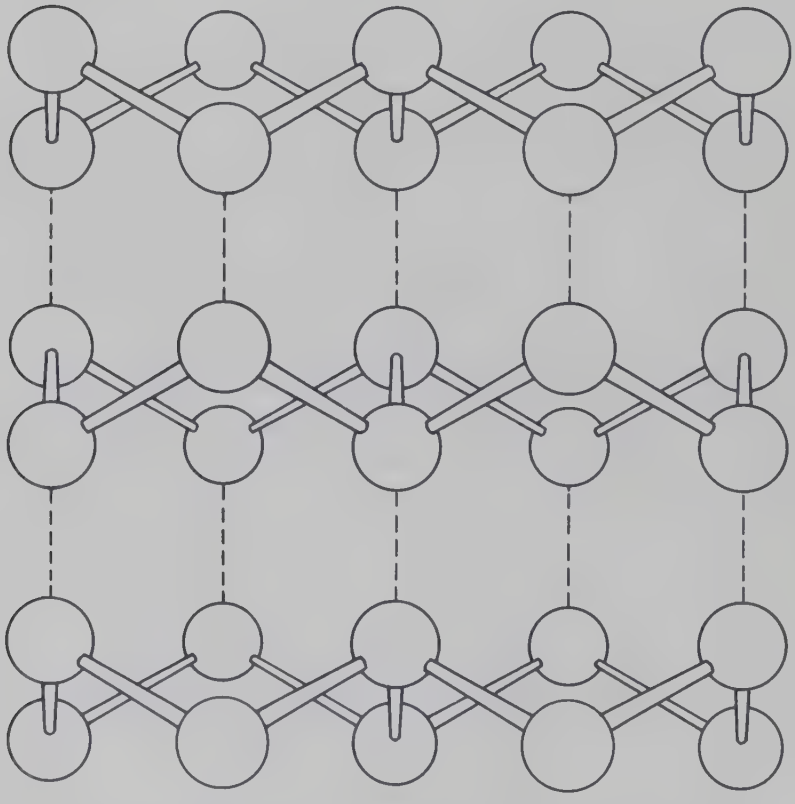

Figure 3. Superimposition of basal planes into a prismatic structure in the ice crystal.

imposed in the manner indicated in Figure 3, into hexagonal prisms or columns, of which the simplest and most typical form is represented in Figure 4. (Some of the prisms are large enough to be seen with the naked eye.)

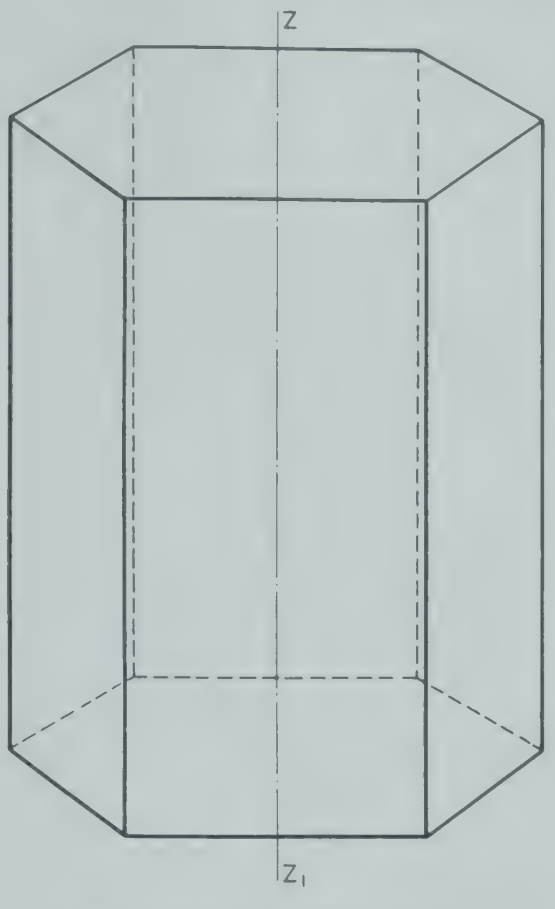

Figure 4. Outline of the typical form of a hexagonal crystal, the hexagonal prism.

A molecule of water measures about 3 angstroms (1 angstrom = 1/250,000,000 inch). Thus, some 3000 molecules in a row are required to cover a length of one micron (1/25,000 inch); 3000 such rows will make a layer one micron square in area; and 3000 layers will make a micron cube. It would then take about 27 billion molecules (3000^3) to construct a crystal of ice large enough to be well observable under the microscope. One billion such blocks (1000^3) would make a cube of ice measuring one millimeter (1/25 inch) on the side (comparable to a fair-size pin head).

Since a cube of ice of those dimensions may readily be constructed in one second, one may imagine the titanic activity that one would witness if he were able to observe the assembling of the molecules in that construction. At a magnification of a million, a molecule of water would have the size of about one seventieth of an inch, and the 1/25 inch ice cube would have sides measuring about 1100 yards. Before freezing, it would be a droplet of water seen as a mound of construction materials covering more than a square mile. In one second we would see that enormous pile of building blocks become transformed into a finished monument. And all that the architect would have had to do would have been to touch the pile of materials at one place with a cold rod (process known as ice seeding or nucleation). That truly magic touch would have triggered into action the molecules which possessed in themselves the power of moving into their proper position in the structure.

Let us now see what happens if, instead of a lake of water, we have a lake

of solution, say, of sugar, or of gelatin, or of albumin. The problem is essentially that of finding how the formation of ice is hindered by the presence of the foreign molecules interspersed through the molecules of water.

II. ICE FORMATIONS IN AQUEOUS SOLUTIONS

Material and Methods. Solutions of some twenty colloids and crystalloids in particular, gelatin, albumin, myosin, sucrose and glycerol, were examined (separately).

Thin layers of solution, sealed between two cover glasses, were frozen by immersion in baths at various temperatures from near zero to -150° C. and the crystalline formations obtained were photographed through a microscope, in an apparatus especially constructed for that purpose (1,2). The principal features investigated were the patterns of ice formation and their rate of development and of growth; and the main variable factors: the nature of the solute, its concentration, the temperature of the bath, and concomitantly with the latter, the cooling velocity.

Results. A broad variety of crystalline forms was observed, which can be classified, according to the degree of order, the mode of arrangement of their component parts and other particularities of structure and of growth, into the following six types, illustrated in the photographs of Figure 5: 1. Hexagonal forms (Photograph 1); 2. Irregular dendrites (Phot. 2); 3. Lobed formations (Phot. 4); 4. Spherulites (Phot. 3); 5. Evanescent spherulites (Phot. 5); 6. Recrystallization clouds (Phot. 6). The first five can be seen to grow, upon cooling of the solution, from centers of crystallization; the sixth is the result of growth, upon rewarming, of diffusely distributed crystalline nuclei formed in the course of the previous cooling.

Which of the first five types of crystalline formations are obtained, in the course of cooling, depends largely on the degree of disturbance of the crystallization process. The main causes of disturbance are a high freezing velocity and the presence of molecules of solutes, in particular of long and stringy ones, as is the case with gelatin (Phot. 4 of Fig. 5).

The general effects exerted by these factors can be summarized as follows: When the molecules of water are allowed sufficient time, they arrange themselves in an orderly fashion into crystals which show the typical hexagonal symmetry (Phot. 1, Fig. 5); when they are not allowed enough time, the temperature being too low, or when their movements are hindered in various ways, they attach themselves to the first comers, and this results in the various patterns represented in Photographs 2 to 5 of Figure 5, in which one can distinguish several steps in the loss of regularity (3).

III. ICE FORMATION IN THE FLUIDS OF MUSCLE

Materials and Methods. Fresh frog's muscle was ground in a "Waring blender," and centrifuged; the supernatant fluid was then frozen, either at its natural concentration or after the concentration had been increased by dehydration.

Results. The extracted fluids gave the ice patterns displayed by most solutions at equivalent concentrations and temperatures, namely, hexagonal forms, irregular dendrites and evanescent spherulites. Upon being rewarmed the regions occupied by evanescent spherulites darkened into recrystallization clouds. Lobed formations, such as those reported with solutes consisting of long, stringy molecules (gelatin, polyvinyl pyrrolidone), and coarse spherulites, such as those commonly found in the presence of small molecules (glycerol, sucrose) did not appear in our muscle juice preparations. Thus, the

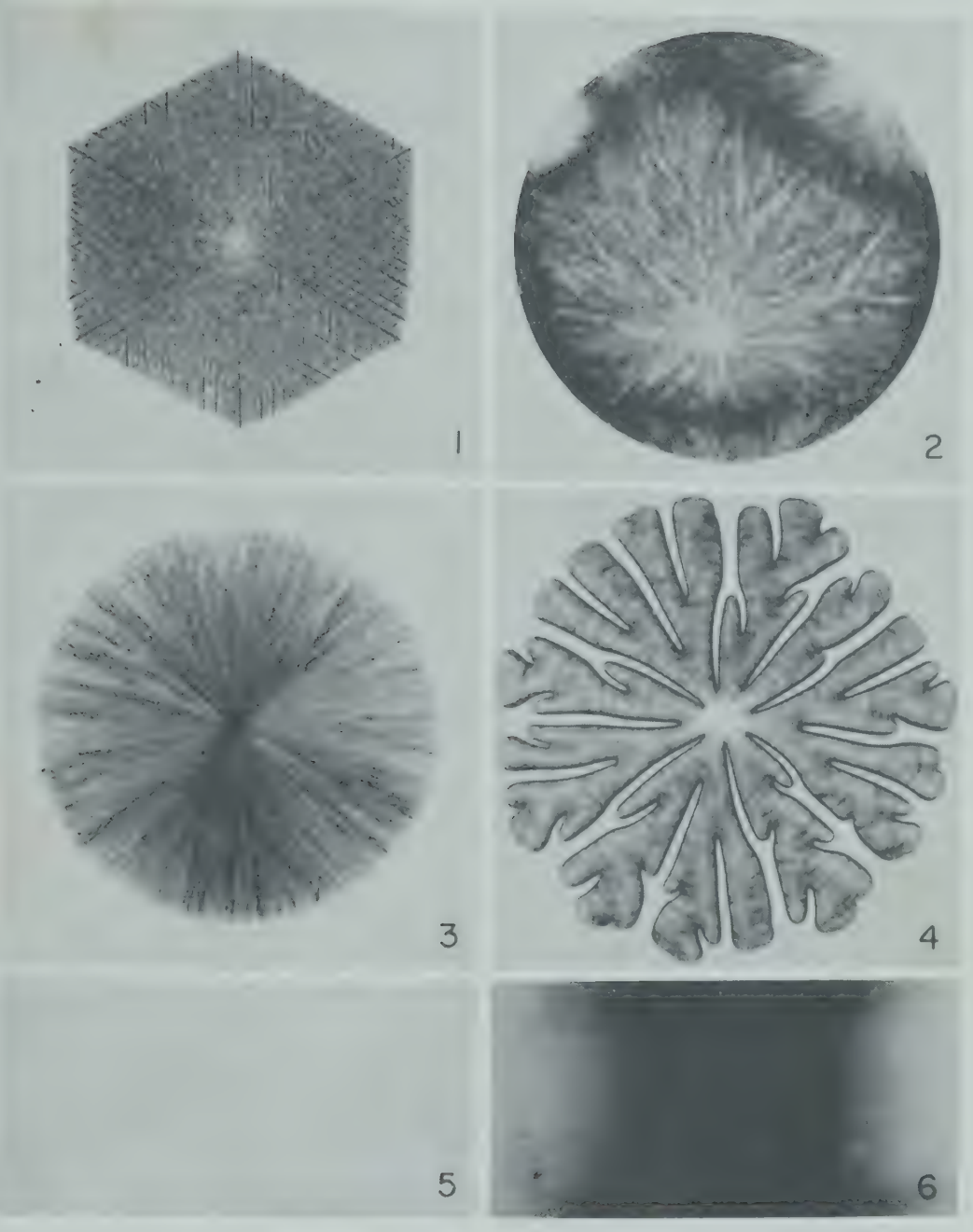

Figure 5. The six main types of ice formations encountered in aqueous solutions at various solute concentrations and temperatures. Photograph 1: Hexagonal form, obtained in 6 M glycerol at -35° C.; Phot. 2: Irregular dendrite, 10% sucrose, -65° C.; Phot. 3: Spherulite, 6 M glycerol, -60° C.; Phot. 4: Lobed formation, 50% gelatin, -25° C.; Phot. 5: Evanescent spherulite, 50% sucrose, -45° C.; Phot. 6: Recrystallization cloud, 30% gelatin, -30° C. Magn.: 71.25 X. (From Luyet and Rapatz, Bibl. Ref. No. 3.)

structureless components of muscle behaved like solutions containing solutes of relatively high molecular weight. I shall now examine the effects of the structure of intact muscle tissue on the development and growth of ice.

IV. MECHANISM OF ICE FORMATION IN MUSCLE

Introduction: Molecular Anatomy of Muscle

To picture a muscle as it would be seen at the molecular level, we need to magnify it about a million times. At that magnification, the molecules of water would appear—as was already said—as particles measuring about one seventieth of an inch (0.3 mm.). These molecules (w in Insert C of Fig. 6) would be seen to surround or penetrate parallel rods of actin or myosin (ms in Fig. 6), the myofilaments; these would have a diameter of 1/10 to 4/10 inches (25 to 100 angstroms) and be from one to two inches apart. The myofilaments are arranged into bundles of hundreds to a thousand, the myofibrils (fl in the figure), which would appear to be some 40 inches across (1 micron). The myofibrils are themselves assembled into bundles ten to fifty times larger, the fibers; these are enclosed in a membrane, the sarcolemma (sl). In cross sections, the fibers would appear to be some 10 to 50 yards in diameter (10 to 50 micra). The muscle itself consists of a large number of parallel fibers. (Figure 6 shows a small portion of a fiber, consisting of two fibrils (fl), in cross section in A and in longitudinal section in B. It also shows the relative size of the myosin filaments (ms) and of the molecules of water (w), at a magnification 10 times higher, in Insert C, and the relative size of the fibers in a muscle, at a magnification a hundred times lower, in Insert D.)

Seen as an entire unit, a spindle-shaped muscle, one inch long and one tenth of an inch in diameter in its center, would appear, at a magnification of a million, as a structure some 15 miles long, and 1.5 mile in diameter in its center, consisting of threads or wires (the myofilaments), arranged parallel into cables (the fibrils); these, in turn, are arranged into larger bundles (the fibers), themselves arranged into a giant, spindle-shaped bundle (the muscle). The entire structure is bathed in a lake of water and is penetrated by water molecules seen as fine grains of sand.

Ice Formation in Muscle Fibers

Material and Methods. All the experiments reported here were performed with single muscle fibers teased from frog's skeletal muscles. In one series of experiments (described hereafter under (a) the fibers had their full water content; in another (b) they were slightly dehydrated.

(a) The fibers, teased in paraffin oil, were mounted between two cover glasses, frozen at various low temperatures in the manner described above for layers of solution, and photographed or cinematographed. At temperatures below -15° C. seeding was generally spontaneous; at higher temperatures it was obtained by contact of the preparation, toward the tip of the fiber, with a cold rod.

(b) The fibers, teased in Ringer's, and touched with blotting paper for the removal of the excess fluid, were exposed for two minutes in a moist chamber at about 94% relative humidity, where their water content decreased to about 70%. Then they were mounted between cover glasses and handled as described under (a).

Some of the frozen fibers were sectioned with a microtome in a room at -30° C., and some were dried by vacuum sublimation, embedded in paraffin and sectioned (4).

Results and Discussion. In cross sections of frozen and dried fibers

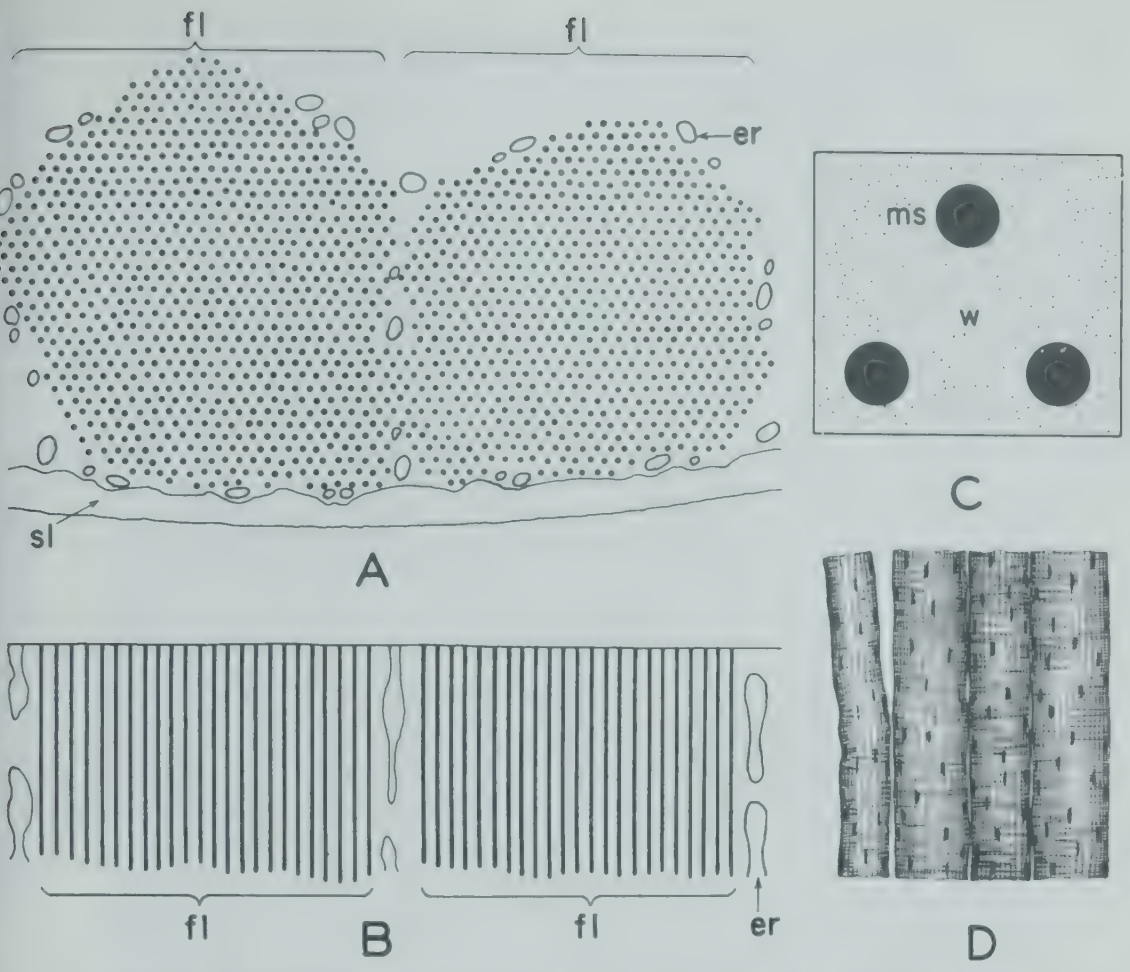

Figure 6. Diagrams representing the structure of a muscle fiber. A. Cross section through a small fraction of a fiber, showing two fibrils (fl) with their myosin filaments, the endoplasmic reticulum (er) and a small portion of the membrane surrounding the fiber, the sarcolemma (sl). Magn.: 60,000 X. B. Longitudinal section through the fraction of the fiber represented in A. C. Portion of A enlarged 10 times to show the relative sizes of the myosin filaments (ms) and the molecules of water (w). D. A group of fibers drawn at a magnification 100 times lower than the magnification used for A and B.

there are holes of more or less circular shapes (Fig. 7, Photograph 1), and in longitudinal sections, elongated, oval or spindle shaped spaces (Phot. 2), or long and narrow channels (Phot. 3). These being the loci previously occupied by ice, it appears that the fibers have been invaded by ice columns which developed preferentially in the longitudinal direction and which, in their formation, had pushed aside the solid constituents of the fibers.

That this is, in fact, the mode of invasion can be established by direct observation or by means of motion pictures, when the rate of freezing has been slowed by partial dehydration and by the use of high, sub-zero freezing temperatures. The ice columns have the shape of spears (Fig. 8, Phots. 1, 2 and 3) and their pointed ends are seen making their way through the fiber in the longitudinal direction. One cannot, however, ascertain from these pictures whether the spears separate intact fibrils or forge ahead through them.

In the case illustrated in Photographs 1, 2 and 3 of Figure 8, the pointed end of the ice column advanced at the rate of about 2.5 micra per second and its diameter reached 8 micra in 40 seconds and 10 micra in 80 seconds, after

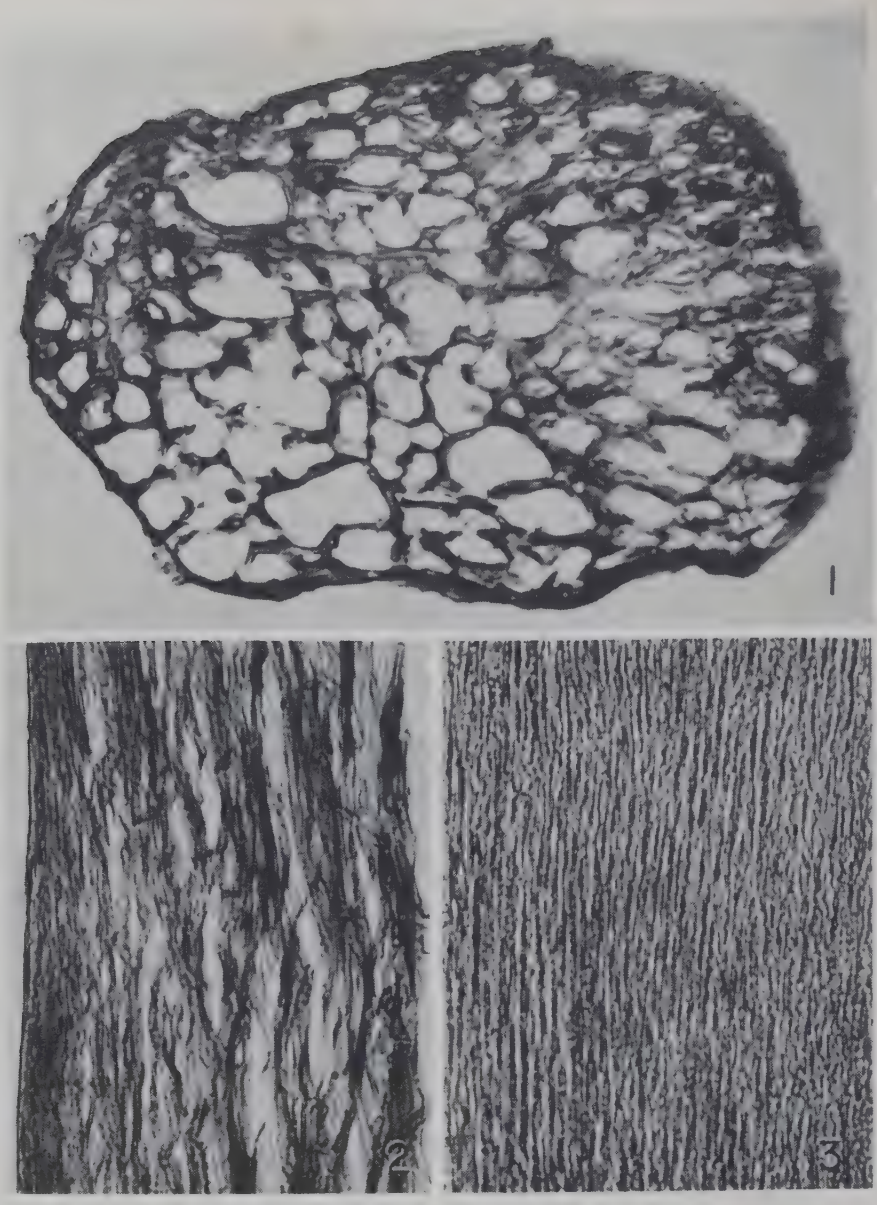

Figure 7. Cross section (Phot. 1) and longitudinal sections (Phots. 2 and 3) of single, partially dehydrated, freeze-dried muscle fibers. Freezing temperatures: Phots. 1 and 2, -15° C.; Phot. 3, -70° C. Magn.: 382.5 X. (From Rapatz and Luyet, Bibl. Ref. No. 4.)

which growth was very slow. Apparently the myofibrils and myofilaments exert a resistance to the transversal growth of the ice columns which laboriously attempt to extract water from them, whereas the interfilamentous or interfibrillar spaces offer a relatively free passage in the longitudinal direction.

As a result of the enormous difference in speed in the two directions, the ice phase appears to develop almost only longitudinally.

In fibers with full water content, ice spears are seen to "shoot" at high speed in the longitudinal direction, even at high freezing temperatures. Radially the spears form buds (Fig. 8, Phots. 4 to 6) which, at slightly lower freezing temperatures, spread out vigorously (Fig. 8, Phots. 7 to 9) and may soon occupy the major part of the diameter of the fiber.

The number of ice columns formed in a fiber at a given water content increases, in general, at lower freezing temperatures, and their diameter decreases (cf. Fig. 7, Phots. 2 and 3). This observation is merely an extension

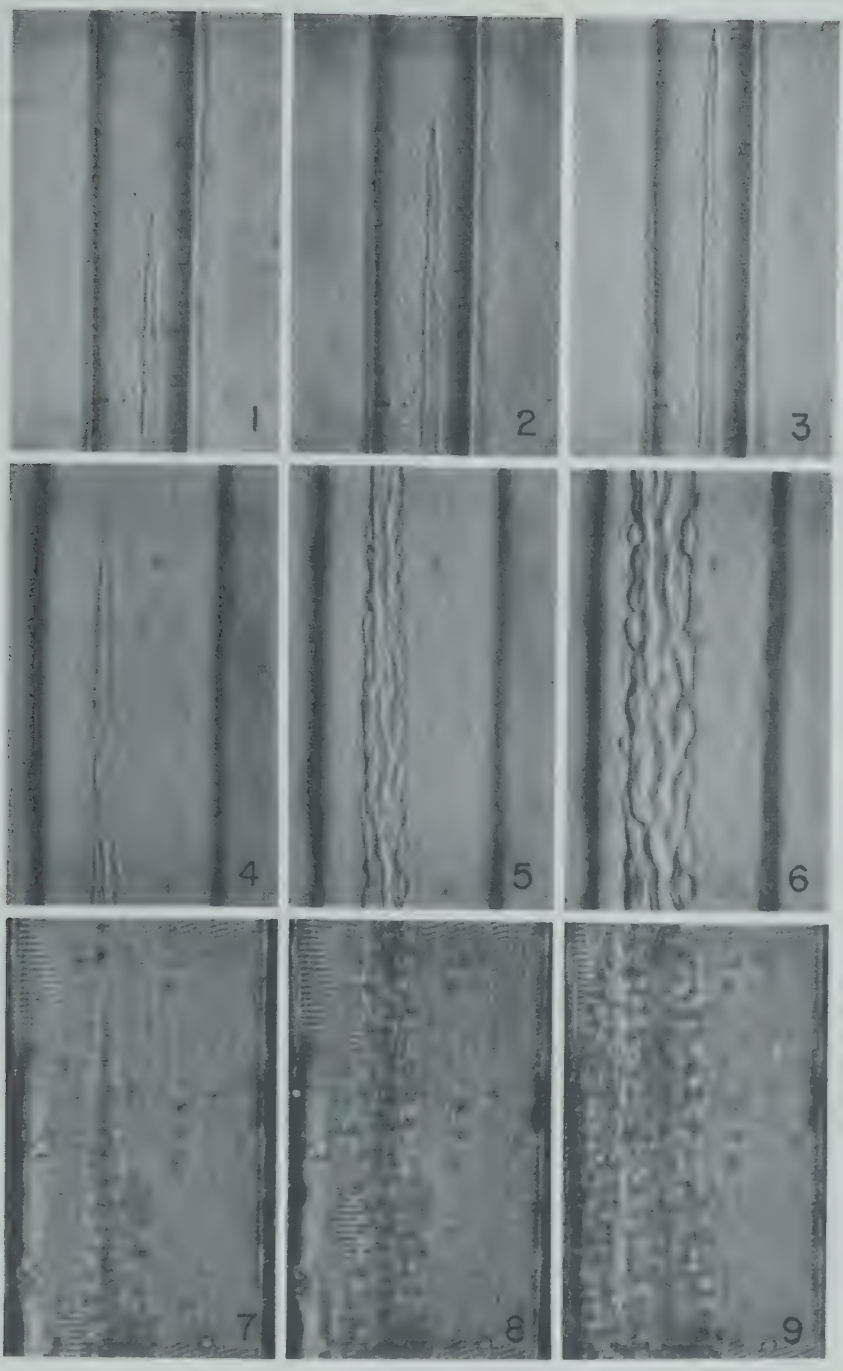

Figure 8. Motion pictures showing the development and growth of ice spears within single muscle fibers. Phots. 1 to 3: Partially dehydrated fiber; freezing temp. -4° C.; pictures taken 2 seconds apart. Phots. 4 to 6: Non-dehydrated fiber; freezing temp. -2.5° C.; showing the development of protuberances around the spears. Phots. 7 to 9: Non-dehydrated fiber; freezing temp. -3° C.; showing the vigorous growth of the protuberances. Magn.: 232.5 X. (From Rapatz and Luyet, Bibl. Ref. No. 4.)

to single fibers of the observation made by previous investigators that the ice masses formed in a piece of muscle tissue are larger at lower cooling velocities (5,6). It is also, of course, a mere extension of the general principle that lower freezing rates permit the formation of larger crystals.

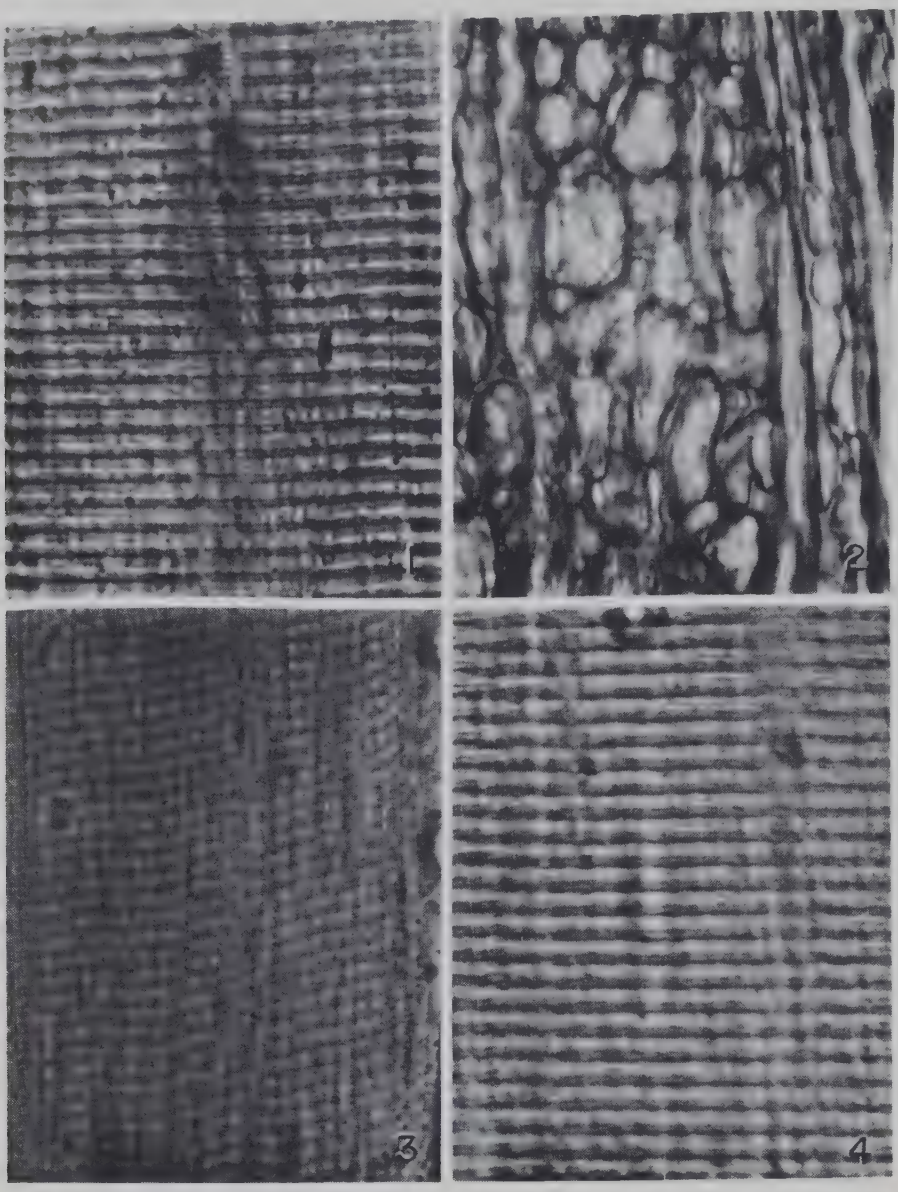

Figure 9. State of preservation of striations in single, non-dehydrated muscle fibers. Phot. 1: Control, non-frozen fiber. Phot. 2: Fiber frozen at -2.5° C. Phot. 3: Fiber frozen at -40° C. Phot. 4: Fiber frozen at -2.5° C. and thawed. Magn.: 750 X. (From Rapatz and Luyet, Bibl. Ref. No. 4.)

The types of ice patterns observed or indicated include: (1) Spears, as described; (2) Veils of incipient crystallization moving longitudinally through the fibers; (3) Clouds formed upon warming over the areas occupied by the veils. These three modes of crystallization appear to be representative forms of the three crystalline patterns described above under the names of, respectively, irregular dendrites, evanescent spherulites and recrystallization clouds, and represented in photographs 2, 5, and 6 of Figure 5. That is, the center of an irregular dendrite (as that of Phot. 2 in Fig. 5), or the center of

an evanescent spherulite (as that of Phot. 5) would begin to grow at the seeded end of a fiber, but the tubular structure of the fiber and its filamentous content would permit only one branch of the dendrite to grow into a spear, or one sector of the spherulite to grow into a veil, within the fiber. Recrystallization of the area covered by the veil would render it opaque, in the manner shown in Photograph 6 of Figure 5.

Since I intend only to point out here the fact that some of the patterns of crystallization obtained in structureless solutions are recognizable in tissues, I shall not elaborate further on those problems, but will refer the reader for more details to the original papers by Luyet and Rapatz (3) and by Rapatz and Luyet (4).

Now that we have a little more insight on the mode of ice formation in muscle fibers, we are in a better position for evaluating the damage done by freezing. I shall limit myself here to the damage recognizable by the condition of the striations. The photographs reproduced in Figure 9 illustrate the essential points: (1) Relatively slow freezing, with formation of ice masses or ice columns which cover large portions of the fiber, distorts the contents of the latter to such an extent that the striations disappear. (2) Very rapid freezing, leading to ice columns of the order of a micron, still leaves the striations in a fair state of preservation (Phot. 3). (3) Striations which have been obliterated by freezing (to the extent shown in Phot. 2) reappear upon thawing (Phot. 4); they evidently have been preserved in the compressed fibrils, as they appear almost normal after the fibrils have reimbibed water (compare Phot. 4 with Phot. 1, which represents an untreated fiber).

Our next step in this research is to proceed from the level of the fiber to that of the fibril, that is, to study the mechanism of freezing within a fibril, and the behavior of ice toward the myofilaments. This we are now doing with the electron microscope.

I wish to point out, finally, that some of the features of the mode of ice formation described may be particular to the procedure followed.

ACKNOWLEDGMENT

All the photographs reproduced in this paper were taken by my research associate, Dr. G. Rapatz. The drawings are the work of Mr. Max Persidsky of the research staff of this Foundation.

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VII

THE EFFECT OF CURING SALTS ON BACTERIAL SPORES

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Canned cured meats are merchandized either as perishable or shelf stable products. In both categories the products are heat processed to an extent sufficient to destroy all vegetative forms of food poisoning bacteria. Even in shelf stable canned cured meats, however, the heat treatment given is not sufficient to eliminate all bacterial spores; the process given is necessarily far below that applied to other low acid foods. For example, canned vegetables are prone to spoilage if they are not given a heat process equivalent to about three minutes at 250° F.—expressed as $F_0 = 3$. Cured meats become totally inedible if subjected to that degree of processing. Indeed, severe organoleptic breakdown occurs well below $F_0 = 1$. Subculture of both shelf stable and perishable canned cured meats reveals small numbers of viable aerobic and anaerobic spores. Despite the relative ease with which these spores can be demonstrated in the finished product, the industry has enjoyed a long history of satisfactory commercial production.

One is concerned in canned cured meats, then, with a commercially sterile food, i.e., a product which has been heat processed to an extent sufficient to eliminate vegetative bacteria and has an environment which is unfavorable to the germination and outgrowth of surviving spores. Numerous shelf stable, commercially sterile, non-meat foods are to be found, viz. canned tomato juice and sauerkraut. In these products, the acidity prevents germination and outgrowth of spores. Perishable canned cured meats obviously owe their stability to the practice of merchandizing under refrigeration. With respect to shelf stable cured meats, the most obvious explanation for stability lies in the inhibitory effect of curing salts, NaCl, $NaNO_3$ and $NaNO_2$. As we shall see, however, the presence of curing salts in heat processed cured meats does not fully account for the stability of these products; rather, the presence of these agents in combination with heat shock to the contaminating spores is the key to the stability of canned cured meats.

THE TEST SYSTEM—SHELF STABLE CANNED CURED MEATS

Before attempting a more detailed discussion of the interplay of factors responsible for the stability of these products, it would seem profitable to define more precisely the test system (Table 1).

Many of the studies on the stability of canned cured meats have involved packs inoculated with large numbers of Cl. botulinum spores. Yet, a number of workers have reported that the raw material, red meat, consistently shows a very low order of contamination with putrefactive anaerobic spores (Harriman et al., 1948; Burke et al., 1950; Ayres and Adams, 1953; Brown, 1957; Silliker et al., 1958). Also of significance is the pH of meat (5.6 - 6.2) and its reducing capacity. These factors are of importance with respect to the anti-

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TABLE 1

The Test System
Canned Cured Meat Products

Raw Material:	Uncooked beef and pork Indigenous putrefactive spore population less than one per gram.
Salt:	3-6% brine (no regulation)
$NaNO_3$:	0.0078 – 0.06% (no regulation)
$NaNO_2$:	Not greater than 200 p.p.m.
Processing:	1. Perishable – pasteurization 2. Shelf stable – F_0 less than 1.0.

bacterial activity of nitrite (Tarr, 1941; Henry et al., 1954).

As indicated, the brine content of cured meats varies over a rather broad range. The upper limit of salt in such products is, however, self limiting; products with greater than 3.5% salt (about 6% brine) are considered oversalty by the consumer.

No regulation limits the amount of nitrate that may be added to these products directly. Indirectly, however, the amount of nitrate that may be added is controlled by regulation of the final nitrite level in these products. This may not exceed 200 p.p.m. and, of course, includes nitrite that is formed through the bacterial reduction of added nitrate prior to processing.

As indicated previously, these products are given thermal processing in excess of pasteurization but not sufficient to render them sterile. The surviving organisms are anaerobic and aerobic spores.

SODIUM CHLORIDE

Tanner and Evans (1933) concluded that "salt is the most active preservative used in meat curing solutions." There seems little doubt that in high concentration sodium chloride is capable of preventing completely the germination and outgrowth of bacterial spores and that salt is necessary to the stability of canned cured meats. Just as certain is the fact that organoleptically acceptable levels of salt do not inhibit Cl. botulinum and other spore forming organisms. Selective data in support of this point are seen in Table 2. It will be noted that Tanner and Evans (1933) observed growth of Cl. botulinum in the presence of as much as 10.5% salt. Obviously, this is far above the salt content of merchandizable canned cured meats. Yesair et al. (1944) observed a difference between pasteurized spores and spores which were subjected to a heat treatment approximating F = 0.015. Pasteurized spores were not inhibited by 5% salt, whereas the more severely heated spores failed to grow in the presence of 3.5% salt. Stumbo et al. (1945) on the other hand observed growth of putrefactive anaerobic spores in meat which had received heat treatment in excess of F_0 = 1.0; Silliker et al. (1958) observed growth of surviving indigenous spores in meat containing 3.5% brine after processing to F_0 = 0.13. The work of Yesair et al. (1944) suggests that heat treatment slightly above pasteurization combined with an organoleptically acceptable salt level imposes dormancy on Cl. botulinum spores, but conflicting results of other workers warrant the conclusion that salt alone does not confer stability. Recent work

with pasteurized Cl. botulinum spores in cured meats containing various amounts of salt has indicated that growth and toxin formation may be masked at high brine levels. At 7.1% brine 27 out of 201 apparently normal cans of comminuted beef were found to contain toxin (Greenberg et al., 1959). These findings suggest that high levels of salt impose a hazard, since the usual evidences of spoilage are masked.

TABLE 2

The Effect of Salt on the Growth of Putrefactive Anaerobes in the Absence of Other Curing Agents

Reference	Test System	Results
Tanner and Evans - 1933	Pasteurized spores in culture medium	Growth of Cl. botulinum in as high as 10.5% salt
Yesair, Bohrer, Cameron - 1944	Pasteurized spores in culture medium	Growth of Cl. botulinum in 5% salt but not in 10%
Yesair, Bohrer, Cameron - 1944	Spores treated at $F_0 = 0.015$* in culture medium	No growth of surviving Cl. botulinum spores in 3.5% salt
Stumbo, Gross, Vinton - 1945	Spores treated in meat given $F_0 = 1.0$ and more	Growth of putrefactive anaerobes at 5.5 - 6% brine*
Bulman and Ayres - 1952	Pasteurized spores in meat	Growth of putrefactive anaerobes at 7.5% brine*
Silliker, Greenberg, Schack - 1958	Spores treated in meat given $F_0 = 0.13$	Growth of putrefactive anaerobes at 3.5% brine
Greenberg, Silliker, Fatta - 1959	Pasteurized spores in meat	Botulism toxin formation in 7.1% brine

*Calculated from author's data.

SODIUM NITRATE

Jensen (1944) wrote: "In the field of microbiology of meats, there are few subjects over which there has been more controversy than on the effect of sodium nitrate on anaerobic bacteria in meats." Richardson (1907) attributed to sodium nitrate the ability to create aerobic conditions in an environment which is anaerobic in the bacteriologic sense. Jensen and Hess (1941) reported the results of experiments involving the stability of pasteurized meat cured with and without nitrate. Their conclusions were that nitrate in cured products is of benefit in preventing the growth of putrefactive anaerobic bacteria.

A critical examination of the literature fails to lend support to Richardson's hypothesis. The data summarized in Table 3 clearly indicate that putrefactive anaerobic bacteria are capable of growth in the presence of amounts of nitrate far in excess of that which is used in cured meats. It is of interest to note that Bulman and Ayres (1952) found that anaerobic spores had about the same tolerance for sodium nitrate as for sodium chloride. Yesair et al. (1944) reported inhibition of Cl. botulinum in culture media which were given a heat

TABLE 3

The Effect of Nitrate on the Growth of Putrefactive Anaerobes

Reference	Test System	Results
Leifson - 1931	Pasteurized spores in culture media	Growth in 3% $Ca(NO_3)_2$
Tanner and Evans - 1933	Pasteurized spores in culture media	Toxin formation by Cl. botulinum at 4.4% $NaNO_3$
Jensen and Hess - 1941	Pasteurized meat	Growth of Cl. sporogenes in 0.25% $NaNO_3$ in presence of about 5.5% brine
Yesair, Bohrer, Cameron - 1944	Pasteurized spores in culture media	Growth of Cl. botulinum in presence of 0.25% $NaNO_3$
Yesair, Bohrer, Cameron - 1944	Spores receiving F_0 = 0.045 in culture media	Inhibition of Cl. botulinum by 0.1% $NaNO_3$
Stumbo, Gross, Vinton - 1945	Spores in meat receiving $F_0 > 1.0$	Growth of putrefactive anaerobes in presence of 0.18% $NaNO_3$
Bulman, Ayres - 1952	Pasteurized spores in meat	Growth of putrefactive anaerobes in presence of 3.5% $NaNO_3$
Silliker, Greenberg, Schack - 1958	Spores in meat receiving F_0 = 0.13	Growth of putrefactive anaerobes in presence of 0.0156% $NaNO_3$

process approximating F_0 = 0.045 in the presence of 0.1% sodium nitrate. Pasteurized spores grew in the presence of 0.25% sodium nitrate. Stumbo et al. (1945) and Silliker et al. (1948) failed to observe inhibition of anaerobic spore growth in meats containing nitrate, even though heat processes in excess of that reported by Yesair et al. (1944) were studied.

Jensen (1934) wrote "It is an easily proved fact that nitrate-nitrite-salt-sugar cure favors most phases of aerobic bacteria, whereas nitrite-salt-sugar cure always inhibits fermentation and aids putrefaction." Our own work confirms these conclusions in part (Silliker et al., 1958). Table 4 summarizes data from experiments in which comminuted meat with and without nitrate was subjected to heat prccessing slightly in excess of pasteurization. It will be noted that the addition of nitrate stimulated the appearance of non-putrid swell. The responsible organism in these cases was a member of the genus Bacillus. It is of interest to note, however, that in the absence of both nitrate and nitrite non-putrid, Bacillus-induced swells occurred (note results with 5% brine and control variables). Further, in this series the only instance of putrefactive swelling was in the cure variable containing 3.5% brine together with nitrate.

In the Jensen paper (1934) it is stated that all the aerobic bacilli isolated from swollen cans of chopped cured ham were nitrate reducers. It would seem that a more logical explanation for the activity of nitrate, relative to organisms in the genus Bacillus, would lie in the position of nitrate as a hydrogen accept

TABLE 4

Effect of Individual Curing Ingredients on the Shelf Stability of Canned Meat. F_o: 0.08 — 0.13

Cure	Total Cans Packed	Non-Putrid Swells	Putrid Swells	Sound Cans	Percentage of Pack Sound
5% Brine	12	4	0	8	67%
5% Brine + Nitrate*	12	11	0	1	9%
5% Brine + Nitrite†	12	0	0	12	100%
5% Brine + Nitrate + Nitrite	12	0	0	12	100%
3.5% Brine	12	6	0	6	50%
3.5% Brine + Nitrate	12	9	2	1	9%
3.5% Brine + Nitrite	12	0	0	12	100%
3.5% Brine + Nitrate + Nitrite	12	0	0	12	100%
0 Control	12	3	0	9	75%

*Sodium nitrate concentration: 0.0156%.

†Sodium nitrite concentration: 0.0078%.

Hence, with the addition of nitrate in an environment free of gaseous oxygen, the nitrate reducing Bacillus is able to carry out fermentation of sugar utilizing nitrate as a hydrogen acceptor. Support for this argument may be found in the work of Bulman and Ayres (1952) wherein concentrations from 0 to 4.5% nitrate were tested in ground pork inoculated with PA 3679. The product received a pasteurizing cook. Typical putrefactive spoilage occurred in tubes containing no nitrate and in those containing 3.5%. Certain tubes underwent a spoilage described as atypical. This was characterized by a change in color to gray-green, much gas production, no foul odor, and no extensive putrefaction. This spoilage did not occur in the absence of nitrate but was observed in the presence of 3.5, 4.0, and 4.5% sodium nitrate. The atypically spoiled meat contained large numbers of aerobic bacilli, and chemical analysis indicated the presence of 2000 p.p.m. of sodium nitrite. No nitrite was found in meat showing typical putrid spoilage nor in sound tubes.

It now seems apparent that nitrate is relatively inert with respect to putrefactive anaerobic bacteria but allows the development of nitrate reducing bacilli in environments free of gaseous oxygen. Thus, any cured meat product containing nitrate should be relatively less stable than the same product in the absence of nitrate, the degree of instability being determined by the amount of

contamination of the meat with organisms capable of utilizing nitrate as a hydrogen acceptor. The concept that nitrate produces an aerobic environment (Richardson 1903; Jensen, 1934) is true only to the extent that nitrate can for a particular group of organisms serve the same function as gaseous oxygen. The concept that nitrate prevents the development of anaerobic bacteria appears to be without foundation.

SODIUM NITRITE

Much of the published information on the antibacterial activity of nitrite is not applicable to an understanding of the stability of canned cured meat products, since much of the early work failed to take into account the profound effect of pH. Tarr (1941) showed that nitrites are particularly active against bacteria only at pH values below 7. Tarr's findings have been confirmed by Henry et al. (1954), Castellani and Niven (1955), and Eddy and Ingram (1956). In addition to pH, the ability of the test system to effect the reduction of nitrite to nitric oxide affects the activity of added nitrite (Henry et al. [1954]). Related to this is the reported greater toxicity of nitrite under anaerobic conditions (Castellani and Niven, 1955).

Table 5 summarizes representative data on the toxicity of nitrite against putrefactive anaerobic bacteria. It is apparent that at the levels of nitrite that are found in canned meats, this agent alone cannot confer stability.

TABLE 5

The Effect of Nitrite on the Growth of Putrefactive Anaerobes

Reference	Test System	Results
Tanner and Evans - 1934	Pasteurized spores in culture media. Alkaline	Growth of Cl. botulinum in presence of 0.39% $NaNO_2$
Jensen and Hess - 1941	Pasteurized meat	Growth of Cl. sporogenes in presence of 0.03% $NaNO_2$
Tarr - 1942	Spores and vegetative cells in culture media. pH 5.6	Inhibition of Cl. botulinum at 0.02% NaNO
Yesair, Bohrer, Cameron - 1944	Pasteurized spores in culture media. Alkaline	Growth of Cl. botulinum in presence of 0.02% $NaNO_2$
Yesair, Bohrer, Cameron - 1944	Spores receiving F_0 = 0.045 in culture media. Alkaline	Inhibition of Cl. botulinum in presence of 0.005% $NaNO_2$
Bulman and Ayres - 1952	Pasteurized spores in meat	Growth of putrefactive anaerobes in presence of 0.08% $NaNO_2$
Silliker, Greenberg, Schack - 1958	Spores in meat receiving F_0 = 0.13	Growth of putrefactive anaerobes in presence of 0.008% $NaNO_2$

MIXED CURES

From the previous sections one is forced to conclude that no single curing ingredient at the concentration that it is used in canned meats is capable of preventing the germination and outgrowth of bacterial spores. We shall now consider the possibility that the effects of these agents are additive and that combinations of salt, nitrite, and nitrate can, under proper conditions, impose stability.

Tanner and Evans (1934) found that no combination of nitrate, nitrite, and salt (at the concentrations in which these agents occur in canned meats) is capable of inhibiting the growth of Clostridium botulinum and other putrefactive anaerobes. Yesair et al. (1944) reported that 0.0156% nitrite combined with 3.5% salt completely inhibited the growth of pasteurized Cl. botulinum spores in culture media. Nitrate at levels of 0.172 and 0.188% in combination with 3.5% salt did not inhibit growth. The addition of 0.172% nitrate to the curing system containing nitrite and salt resulted in delayed growth of Cl. botulinum. Bulman and Ayres (1952), working with pasteurized pork inoculated with PA 3679, found that no combination of curing agents would suppress indefinitely the germination and outgrowth of spores. The combined effects of the agents were, in general, greater than the individual effects. The active agents were sodium chloride and sodium nitrite. Sodium nitrate had little activity. These experiments are summarized in Table 6.

TABLE 6

Mixed Cures: The Additive Effect of Curing Salts

Pasteurized Meat - 50 P.A. Spores/g			
Cure			Result
3.5% NaCl	.125% NO_3^-	.015% NO_2^-	
X			Total spoilage - 2 weeks
	X		Total spoilage - 1 week
		X	Total spoilage - 4 weeks
X	X		Total spoilage - 2 weeks
X		X	Total spoilage - 12 weeks
X	X	X	Total spoilage - 19 weeks
	X	X	Total spoilage - 1 week

From Bulman and Ayres (1952).

It is apparent that whereas mixed cures have an advantage over cures involving the incorporation of a single curing salt, no combination of these agents is completely effective in the inhibition of spoilage. These findings involved spores which had received no more than a pasteurizing heat treatment. Meat products receiving such treatment are purveyed with the instruction "Perishable, Keep Under Refrigeration." Billions of pounds of such product have been sold without a single case of botulism. One would be naive to conclude that this excellent record can be attributed solely to refrigeration, since perishable products are frequently mistreated both on the retail and consumer level. Rather, it appears that this record can be traced, in part, to the low in-

cidence of putrefactive anaerobic spores combined with the tendency of such products to evince organoleptic degradation when they are mishandled.

There are, however, millions of pounds of shelf stable cured meats sold yearly. These products do not spoil even though they are held indefinitely at ambient temperatures. Such products do not receive heat treatment sufficient to eliminate contaminating spores. To what is the stability of these products to be attributed?

THE EFFECT OF HEAT INJURY COMBINED WITH CURING SALTS

A clue to the stability of shelf stable cured meats is found in the work of Yesair et al. (1944). Using pork infusion broth inoculated with Cl. botulinum spores, they tested the effects of curing salts after heat treatment at 185° F. for 1, 2, 3 and 4 hours. The inoculated organisms were recoverable from the tubes after these treatments, but the surviving spores did not grow in all instances. Tubes containing 3.5% salt and heated for one hour were subsequently negative for growth of Cl. botulinum after one year of incubation. Mixed cures were likewise stable, even though surviving organisms were demonstrated on sub-culture. These data suggest that heat treatment slightly above that necessary for pasteurization, combined with the presence of curing salts, imposes upon the spore an injury which renders it incapable of outgrowth in the presence of curing salts. Mr. G. A. Blodgett of our laboratories has computed the F_0 for the processes used in the study by Yesair et al. A treatment for 1 hour at 185° F., assuming no "come-up time," yields an F_0 of 0.0149.

Silliker et al. (1958) studying uninoculated meat processed at F_0 values between 0.08 and 0.13 reported similar findings. Not all curing systems were found capable of producing a stable product. The results summarized in Table 4 show that either 3% or 5% brine combined with 0.0078% sodium nitrite produced a stable product. Neither brine alone nor brine combined with .0156% sodium nitrate was satisfactory. Nitrate increased the incidence of non-putrefactive spoilage over that observed in the absence of nitrate.

It was shown that the stability of these lightly processed cured products is dependent upon a low level of spore contamination. Intentional inoculation of such products with large numbers of putrefactive anaerobic spores resulted in prompt failure of the system. Table 7 shows the results obtained in cured meats receiving an F_0 of approximately 0.1 in the presence of 0.0078% sodium nitrite and 3.5% brine. Both products were inoculated with PA 3679, the first pack at a level of 15,000 per gram and the second, 2.0 per gram. The

TABLE 7

Shelf Stability as a Function of Degree of Contamination

Cure*	Process	Inoculum†	Result
3.5% brine, 0.0078% $NaNO_2$	F_0 = 0.13	15,000/g	66% putrid spoilage, 45 days (100 cans)
3.5% brine, 0.0078% $NaNO_2$	F_0 = 0.10	2.0/g	No spoilage, 90 days (80 cans)

*Mixed with beef-pork emulsion.

†P.A. 3679 spores.

more heavily inoculated pack showed 66% putrid spoilage after 45 days of incubation. The lightly inoculated pack was completely sound after 90 days of incubation. At this time all cans were opened. There was no evidence of putrefaction, and bacteriological analysis showed that although 20% of the anaerobic spores survived the heat process, no growth of these organisms took place during subsequent incubation.

These results confirm those reported by Stumbo et al. (1945) and Vinton et al. (1947). In the earlier paper they reported results on meat tubes inoculated with putrefactive anaerobic spores in the presence of .09% sodium nitrate, .016% sodium nitrite, and 3.5% brine. The tubes received heat processing far above that reported in the studies of Yesair et al. (1944) and Silliker et al. (1958); yet no combination of curing ingredients inhibited the surviving spores. In the 1947 paper, however, uninoculated meat receiving F_0 values of 0.05, 0.2, 0.6 and 1.0 was stable. The authors concluded "That this work should be regarded as preliminary and possibly indicating a promising angle of attack upon the problems related to the thermal processing of canned meat. However, no definite or final conclusion should be drawn because this work should be checked and considerably extended before sufficient data are available to give final conclusions. It is also the opinion of the authors that no attempt should be made to interpret this work as indicating low thermal processes may be commercially safe or that inhibition by salt can be depended upon on a commercial scale to prevent spoilage. A vast amount of work yet remains to be done and to be checked by others on an industry-wide basis before such conclusions are warranted." What seemed to these workers a surprising result in 1947 has now become a fact well tested by commercial meat canners. This fact is that stable canned meats can be processed at levels such that putrefactive and aerobic spores remain in the finished product, provided that the level of contamination is no greater than obtains in commercial meat trimmings. The stability of the product is imposed by sub-lethal heat treatment combined with organoleptically acceptable levels of salt and concentrations of sodium nitrite which fall within legal limits.

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VIII

BACTERIAL PROBLEMS IN PRE-PACKAGED MEATS

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Our studies of pre-packaged meats during the past 12 years have been restricted almost entirely to cured products. The heat treatments applied to most of these products destroys the majority of the bacteria in the raw materials and the curing ingredients plus refrigerated storage severely restrict growth of the survivors. Therefore the types of bacteria that grow in or on cured meats during normal storage are quite different than those growing in pre-packaged fresh meats, as reported, for example, by Dr. Olin Ball and his group last year in Food Technology.

Many pre-packaged cured meats are stuffed into a casing of some kind before they are cooked. If the product is marketed as a whole sausage it is protected by the casing from further contamination. Thus any microbial problems result from the activity of organisms surviving the heat process or from microbial growth on the casing itself. If the casing is removed, however, and the product is re-packaged before sale—as, for example, skinless wieners or sliced-pack items—the surface of the product becomes contaminated to greater or lesser degree with organisms that may grow at normal storage temperatures. Thus opportunities for slime development and surface discolorations appear. We have studied both whole sausages and sliced-packs and find the problems to be quite different.

Whole sausages. Most of our work with this type of product has involved liver sausage and bologna, with emphasis on the first. Sausages stuffed in natural animal casings are susceptible to surface spoilage—that is, growth of microorganisms on the casing itself. In our experience molds, micrococci and a variety of other bacterial types seem to find conditions suitable for growth and become apparent on prolonged storage, especially if the humidity is high. The shelf life of products in natural casings necessarily is limited because of dehydration and chemical discolorations.

With the introduction of artificial casings these problems have been minimized. Loss of moisture from and absorption of oxygen by the product are reduced. Hence there is less shrinkage and loss of normal color. Microorganisms cannot attack the casing itself. But simply because a product is stuffed in an artificial casing does not necessarily mean that it will be free of microbial change (1,2). Table 1 shows results we obtained with liver sausage that was stuffed and cooked in an ordinary transparent cellulose casing and then overwrapped with a rubber hydrochloride film. Bacteria grew in the product immediately beneath the casing and the product lost its normal color. These organisms were identified as species of Lactobacillus. Furthermore, there was abundant growth of a variety of morphological types of bacteria in the water trapped between the casings. Nutrients diffused from the product through the inner casing, thus providing food for the organisms in the film of moisture between the two coverings.

However, when the product was stuffed, cooked and marketed in a single layer of plastic film that had a very low degree of permeability to moisture and oxygen, there was no microbial growth and the product retained its normal

TABLE 1

Bacteria Counts in Liver Sausage Stored at 41° F.

Casing	Plate Count Per Gram*	
	0 Day	78 Days
Double—high permeability	5,200	2,400,000
Single—low permeability	5,200	1,400

*Samples taken immediately beneath casing.

color for weeks.

Some of the plastic films available today make it possible to hold a sausage product for months without appreciable change in weight or color. What happens, though, if the product is not properly refrigerated? Table 2 shows results we have obtained with liver sausage and bologna stuffed in Saran and held at various temperatures (3). Growth was greatly delayed at 51° F. and none was detected at 41° F., but multiplication was fairly rapid at 61° F. The organisms were heat resistant spore formers and micrococci. Even though the counts reached millions per gram, the only evident change in the appearance of the products was a slight softening.

TABLE 2

Effect of Temperature on Growth of Bacteria in Two Sausage Products

Product	Days Storage Without Growth at		
	41°F.	51°F.	61°F.
Liver sausage	>93	>91	13
Bologna	>106	73	12

The excellent preservative qualities of plastic casings with extremely low porosity may be a mixed blessing. Since oxygen absorption and water loss are reduced to an extremely low level, sausage products can be stored in some of these films at temperatures far above normal without gross physical change that would be apparent to a consumer. One cannot help but wonder, therefore, whether there is danger of food poisoning resulting from the growth of heat resistant microorganisms in products that are not properly refrigerated by the packer, the retailer or the consumer.

To test this possibility we have inoculated liver sausage batter with spores of Clostridium botulinum, stuffed it into Saran plastic casings, and cooked it to an internal temperature of approximately 70° C. (158° F.). The sausages then were held at various temperatures and tested for botulinum toxin (4).

At storage temperatures of 18° C. (65° F.) or below, no toxin was detected in three months or more. Thus, even poor refrigeration would seem to offer ample protection against the danger of botulinum poisoning. However, gross mishandling involving storage at room temperature or above allowed the organism to produce toxin within 15 to 60 days. These sausages showed remarkably little loss of normal color. Naturally they were softer than usual and many of

hem showed varying amounts of gas, but the spices covered up any undesirble odors. They would certainly be acceptable to all but the most discriminating and suspicious consumer.

This study clearly showed that liver sausage is a poor medium for Clostridium botulinum and under any conditions approaching normal there is no anger of toxin formation. If the product is grossly mishandled, however, here is a chance of toxin development without other changes that would make he product obviously unfit to eat.

Re-packaged Meats. Much of the beneficial effect of cooking a product nside a casing is lost when the covering is removed and the product is sliced r otherwise handled before re-packaging. The shelf life of such products natrally depends on the nature of the product itself (for example, its brine conentration), the temperature of storage, and the extent of contamination with rganisms capable of growing on the product.

We have stored a variety of sliced products at 34° and 45° F. to observe he changes in bacterial numbers and general appearance. The products were alf-pound units vacuum-packed in Saran film. They showed no appreciable olor changes but some of them did develop obvious sliminess that was apparnt when the slices were pulled apart. Table 3 compares the rates of slime ormation in the four products.

TABLE 3

Effect of Temperature on Spoilage of Sliced-pack Meats

Product	Brine Concentration	Days for Slime to Appear	
		45°F.	34°F.
Vegetable loaf	4.24	20	60
Patties	4.62	20	50
Bologna	4.94	40	>80
Chopped ham	5.76	>80	>80

Immediately after packaging all of these products contained a few hundred to a few thousand bacteria per gram. The rate of growth was directly related to both the brine concentration of the product and the storage temperature. At 45° F., for example, chopped pressed ham, with its high brine concentration, contained only three million bacteria per gram even at 80 days. With the vegetable loaf, however, the count reached 200,000,000 per gram within 3-1/2 weeks. Except for pressed ham the maximum bacterial populations eventually reached were essentially the same at both temperatures, although naturally they were reached much later at 34° F. than at 45° F.

Without exception, the organisms that grew in the four products were salt tolerant lactic acid bacteria, mostly lactobacilli. Because of their low initial numbers they were not detected in the freshly-made products, but they rapidly overgrew the micrococci and sporeformers that survived the heat process. The lactic organisms were not unusually resistant to heat; hence they were likely incidental contaminants introduced during the slicing and packaging operations. Many of them were able to produce a green discoloration when grown aerobically on a slice of wiener. However no green colors appeared within the packaged slices because of the absence of oxygen. A high proportion of the organisms can produce a gummy polysaccharide material when grown on media containing sucrose. In our opinion this fact contributes significantly to the amount

of slime produced when these organisms grow on meats.

One may well wonder what a meat packer can do to minimize bacterial spoilage of re-packaged items and prolong the shelf life of his products. If our results are typical it would appear the only remedy now available is rig sanitation. Lactic acid bacteria grow perfectly well in the absence of free ox ygen. They have a notable tolerance for salt and many of them, if not truly psychrophilic, certainly can grow well at refrigerator temperatures. Furthe more, all we have tested produce considerably more of the gummy polysaccharide when they grow at low temperatures than they do at higher temperatures. In the absence of a chemical inhibitor, therefore, it would appear that a packer's only resource is to keep these organisms out of his product. This means thorough sanitation of equipment, workers' hands and packaging materials used in the slicing and packaging operations.

Fifteen years ago one rarely saw reference to lactic acid bacteria in me products. In the intervening period, however, Dr. Niven's group at the Ameri can Meat Institute Foundation has repeatedly found them to be the cause of green discolorations on cured meats. In our laboratory we have found them a most everywhere we looked. We have demonstrated lactic organisms in almo every sample of meat examined—both cured and fresh. We have found them i stored bacon, fresh pork sausage, fresh beef, pork, lamb, hamburger, wiener and all varieties of cured meats. With rare exception our isolates have not been especially resistant to heat, but the majority of them are tolerant of con siderable salt and can grow well at refrigeration temperatures.

Fortunately, however, these organisms cause little undesirable change when they grow in meat. They may produce a little acid, but a product can ha a tremendous lactic population without noticeable off-flavor. Were it not for the pernicious habit of some of the lactics to cause a green discoloration thei wide distribution might still be unrecognized. Eventually, though, even in the absence of discoloration the lactic organisms will make their presence known if given time. Their apparently senseless habit of converting large amounts o the sucrose sugar into a polysaccharide and dumping this into their surround ings must contribute to the general sliminess of their growth and thus hasten the revelation of their presence.

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IX

COMBINED IRRADIATION-HEAT PROCESSING OF CANNED FOODS*

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The fact that electromagnetic radiations sensitize microorganisms to the lethal effects of heat has been known for a surprisingly long time. Early work on this phenomenon was done with Protozoa. For example, in 1919, Bovie and Kline (1) reported that sublethal doses of ultraviolet light made certain Paramecia heat sensitive. By this they meant that irradiated Paramecia would die quickly at temperatures that were otherwise sublethal for the organism. This work was extended by Bovie and Daland in 1923 (2). Similarly Giese and his coworkers (3,4,5) published a series of papers between 1945 and 1948 in which it was shown that sublethal doses of visible light, ultraviolet light, and X-radiation all increased the sensitivity of Protozoa to the subsequent lethal action of heat. As early as 1938 Curran and Evans (6) showed that irradiation of aerobic bacterial spores with ultraviolet light before heat treatment was always more destructive than heat treatment preceding light.

These early investigators even suggested a reasonable mechanism for the synergistic, lethal action of combined irradiation-heat processing. Their theory probably started with Bovie's report in 1913 (7) that irradiation of egg albumin with ultraviolet light altered the effect of temperature upon the heat coagulation properties of this protein. Since one plausible theory for the lethal action of heat alone relies upon protein coagulation (8), their theory, based upon the synergistic effect of irradiation and heat for denaturing protein, seems to have considerable merit.

It is recognized that there may be some reticence in using results obtained with radiations in the photochemical section of the electromagnetic spectrum for interpreting data obtained with rays from the radiochemical portion. However, the considerable amount of experimental evidence previously quoted strongly suggests that the lethal actions of ultraviolet and ionizing radiations are closely related. Also, this interpretation appears even more tenable if it is postulated that the effect is photochemical in both instances. Such an assumption appears reasonable because ionizing radiations are ultimately degraded to the photochemical range during their passage through matter (9). The difference between ultraviolet and ionizing radiations for sensitizing microorganisms to heat then becomes largely one of difference in penetrating ability.

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With the development of radiation sources that were sufficiently large for industrial purposes, attention was directed towards microorganisms that are important in food spoilage. Thus, in 1954, Morgan and Reed (10) reported results of studies using the thermophilic anaerobe No. 3814, the putrefactive anaerobe 3679 and the acid tolerant flat-sour organisms No. 2253. They used gamma rays and showed that preirradiation sensitized these spores to subsequent heat processing, a finding that was confirmed by Kan et al. in 1957 (18). This extended the knowledge of combined processing to anaerobic bacterial spores and to gamma radiation. In 1955 (11), my colleagues and I showed that this phenomenon applied to the spores of Clostridium botulinum. We further demonstrated that it is possible to reduce the heat processing time for canned ground beef (12,13,14) and canned peas (15) by preirradiation with gamma rays from cobalt-60. This work, however, was exclusively concerned with the microbiological problems involved since we have attempted no organoleptic studies of the products.

Initially, we studied the magnitude of combined processing for killing C. botulinum spores suspended in phosphate buffer, in nutrient broth and in gelatin. For this purpose, the spores were grown, harvested, washed, suspended in selected media, and dispensed into ordinary cotton-plugged culture tubes. These tubes were divided into two test groups. One group was first heat processed and then placed in our large cobalt-60 gamma ray source where portions could be removed periodically for counting the number of viable spores that remained. The other group were irradiated first and then immersed in a boiling water bath. At proper intervals, samples were removed from these tubes for counting.

Results from the first group of tubes are shown in Figure 1. There it is noted that preheating did not sensitize the spores to the subsequent lethal action of gamma radiation. Figure 2 presents results of the second series of tests and shows a sensitization of C. botulinum spores to heat by previous gamma irradiation. It also indicates that the synergistic effect increased with the preirradiation dosage.

From these tests it was concluded that the F_0 value of C. botulinum spores could be reduced at least fourfold by preirradiation with gamma rays from cobalt-60 (11). The magnitude of the effect was less in gelatin and nutrient broth than in phosphate buffer.

With this established, the next step involved testing the combined effect in canned foods. We selected ground beef for study and inoculated packs using C. botulinum spores were prepared. This involved packing preheated ground beef into No. 1 picnic tin cans, setting the can lids loosely in place, and heat-processing for 1 hour with 15 psig. steam. The meat was then inoculated at the approximate geometric center with 1 ml. of a water suspension of C. botulinum spores; the cans were sealed, cooled in ice-water and irradiated with gamma rays from cobalt-60. They were then ready for the heat treatment portion of the process.

A run constituted a group of cans receiving the same irradiation dose. Groups of four cans were heat processed at several F_0 levels for each run and then the cans were incubated at 85° F. F_0 values were calculated from heat penetration records of control cans in each batch. Some of the cans that swelled during incubation were opened and tested for the presence of toxin by mouse inoculation tests. Processes were selected in such a way that no cans swelled in the group from at least one F_0 value. Similar data were taken with raw ground beef. In this instance the methods required modification to the extent that the cans were sealed in a vacuum closing machine. Results are shown for raw meat in Figure 3 and for cooked meat in Figure 4. In both cases, it will be observed that the F_0 required for sterilization was reduced approximately fourfold following irradiation of canned ground beef with 1.3 megarep of gamma radiation.

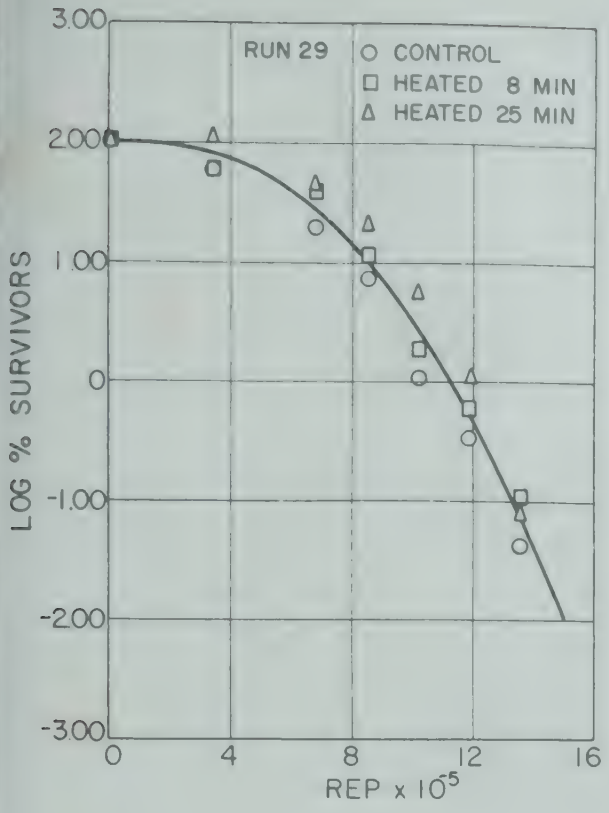

Figure 1. Effect of preliminary heating at 99° C. on the subsequent radiation resistance of C. botulinum 213B spores suspended in 10% gelatin at pH 7.0.

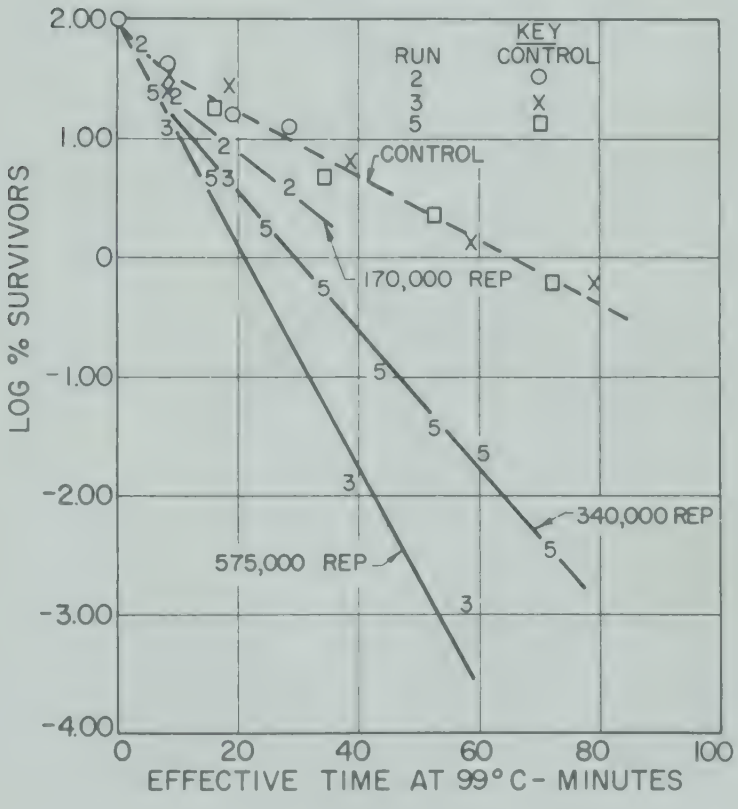

Figure 2. Effect of preliminary irradiation with gamma rays from cobalt-60 on the heat resistance of C. botulinum 62A spores suspended in M/15 phosphate buffer at pH 7.0.

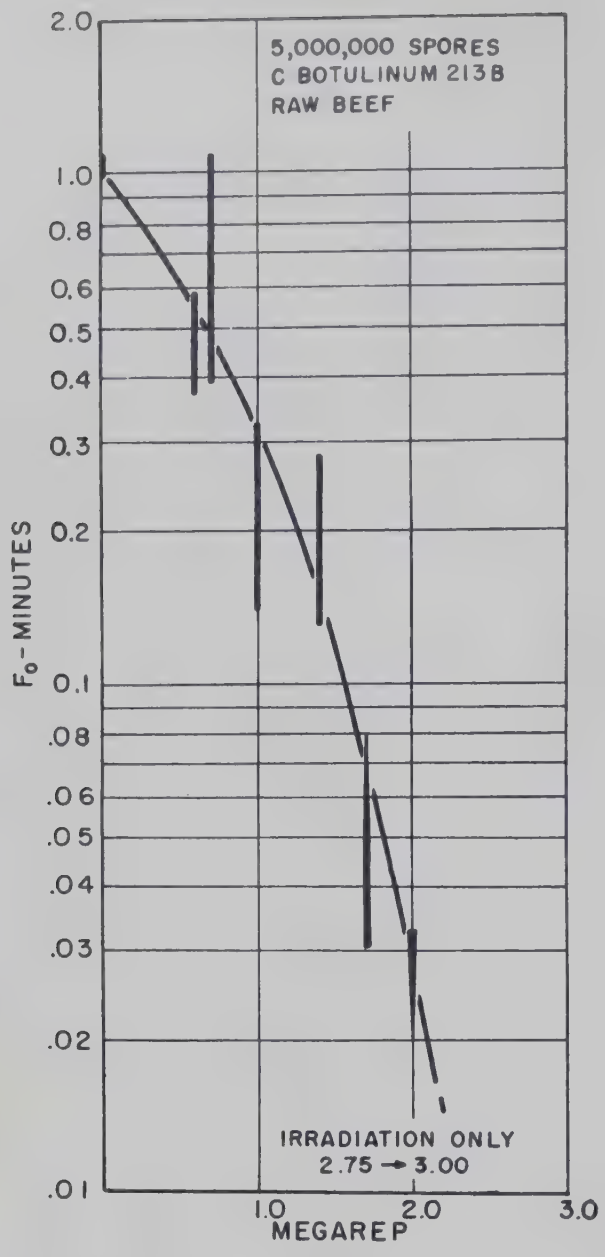

Figure 3. F_0 required to sterilize raw ground beef packed in No. 1 picnic tin cans, inoculated with 5,000,000 C. botulinum 213B spores per can, and irradiated with gamma rays from cobalt-60 before heat processing at 230° F.

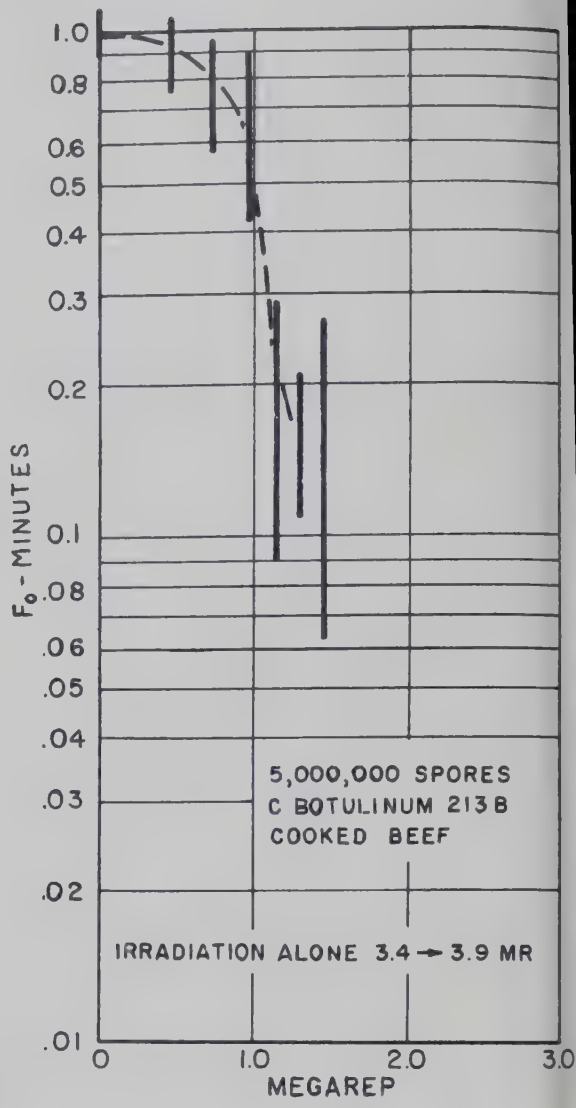

Figure 4. F_0 required to sterilize cooked ground beef packed in No. 1 picnic tin cans, inoculated with approximately 5,000,000 C. botulinum 213B spores per can, and irradiated with gamma rays from cobalt-60 before heat processing.

The question still remained, how would combined processing affect the more heat resistant, PA 3679, type of contaminant? Data from inoculated pack studies in cooked ground beef using spores of this organism are shown in Figures 5 and 6. These results are similar to those previously obtained with C. botulinum spores. They indicate that the F_0 required to produce sterile canned beef following 1.5 megarep of gamma radiation was approximately one fourth of that required when only heat processing was used.

In conclusion, it should be noted that there are other favorable bacteriolog-

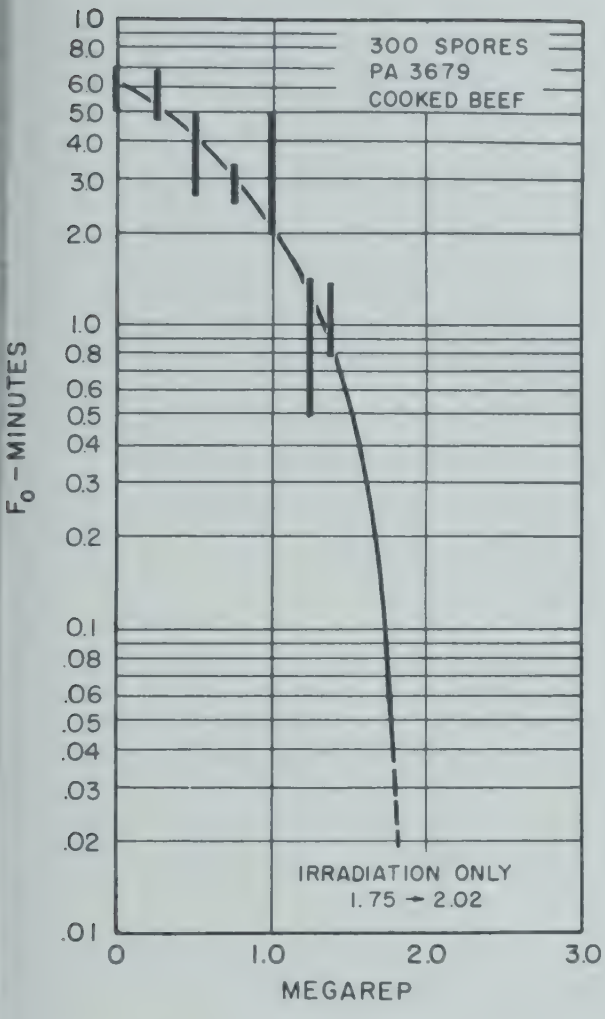

Figure 5. F_0 required to sterilize cooked ground beef packed in No. 1 picnic tin cans, inoculated with 300 PA 3679 spores per can, and irradiated with gamma rays from cobalt-60 before heat processing at 230° F.

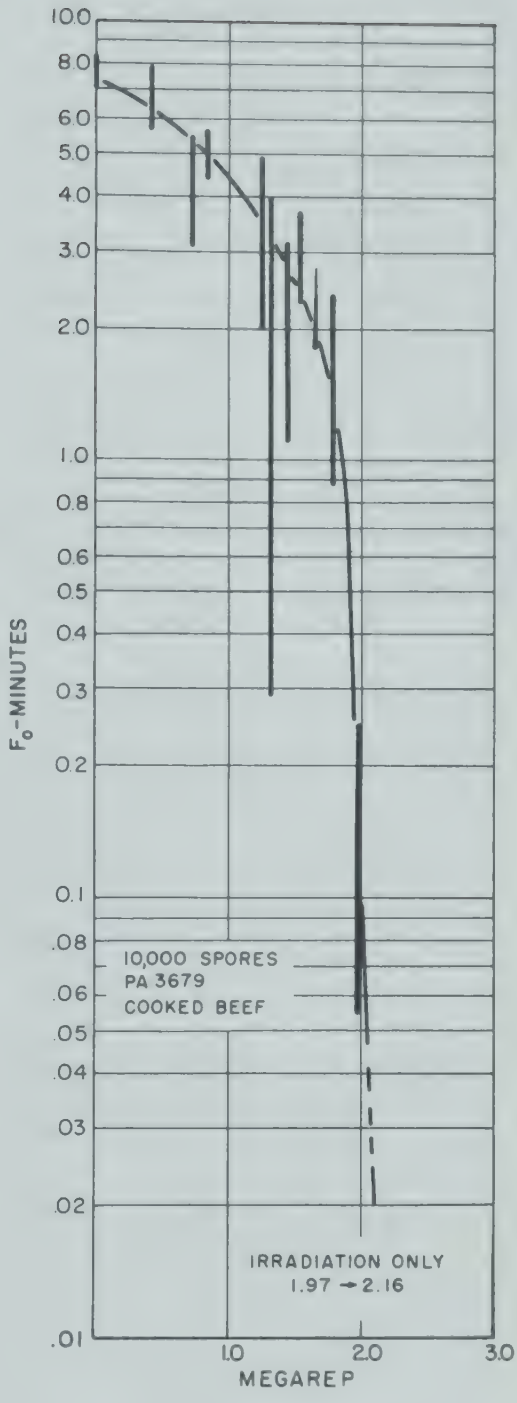

Figure 6. F_0 required to sterilize cooked ground beef packed in No. 1 picnic tin cans, inoculated with 10,000 PA 3679 spores per can, and irradiated with gamma rays from cobalt-60 before heat processing at 230° F.

ical considerations than F_0 reduction which would be characteristic of combined irradiation-heat processing of canned foods. For example, a dosage of 4.5 megarad of ionizing radiations alone may not inactivate all the enzymes, certain micrococci (16), or botulinus toxin that could be present. Except for possibly a few enzymes, these should be destroyed by the heating part of a combined process.

Another bacteriological consideration involves the relative resistance of C. botulinum and PA 3679 spores to heat and to irradiation. In the simple irradiation processing treatment we found that C. botulinum spores are slightly more resistant to irradiation than PA 3679 spores (17); on the other hand, in the combined process, PA 3679 spores are much more resistant. Thus the safety factor present in the usual heat processing treatment, due to the high heat resistance of PA 3679 spores, has been found to be characteristic of the combined irradiation-heat process as well.

Finally, it should be pointed out that my remarks have dealt solely with the bacteriology involved. This is only a basis for the organoleptic studies which need to be carried out to evaluate possible commercial feasibility of combined irradiation heat processing. I do not know whether a fourfold F_0 reduction is sufficient to produce unique or materially improved products. Even the approximately 1.3 megarad preirradiation that appears to be necessary may be sufficient to cause adverse organoleptic changes in foods treated by combined irradiation heat processing. In this connection, however, the fact that the preirradiation portion of the process can be carried out on raw foods could be significant. For example, it might be desirable to irradiate and then heat the foods in open cans for blanching and exhausting purposes before sealing and heat processing. The heating in open cans could drive off volatile "off-flavor" substances produced by the preirradiation. This treatment should not prejudice the subsequent combined processing in any way and could materially reduce possible undesirable irradiation flavors in the final product.

These problems require solution before serious consideration can be given to combined processing for commercial use. However, the possible value of the process should make such research worth the effort.

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X

PACKINGHOUSE WASTE PROCESSING—APPLIED IMPROVEMENTS OF CONVENTIONAL METHODS

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The importance of waste processing for by-product recovery in the meat packing plant has been well established (3,4,5). By-products such as inedible grease and animal tissue represent a large portion of the recoverable products of the industry and loss of these materials can result in heavy pollution loads in the waste waters of the plant. A detailed study of the waste production within the packinghouse will provide basic data as to the criterion of what are good and bad plant operations in the recovery of profitable materials from the waters discharged to the sewers.

During the past several years such a study has been made at The Rath Packing Company in Waterloo, Iowa, and has resulted in several modifications which have reduced excessive losses of valuable materials. The salvage processes which were applied and the results which were obtained are of general interest to the industry.

The plant in which these studies were made slaughters cattle, sheep, and hogs with a slaughtering capacity of approximately 1500 tons per day. Waste treatment facilities within the plant consist of four interceptor basins of approximately 400,000 gallons total capacity. These basins were constructed in 1940 and were designed for primary and secondary sedimentation and grease recovery. At that time these basins provided for two to four hour detention and a flow velocity of two feet per minute. Mechanical flights were provided to facilitate collection of bottom sludge and removal of surface grease. Settled effluent was either pumped through a pressure sewer or allowed to flow by gravity through a second sewer to a municipal disposal plant for final treatment. Clear process waters were discharged directly to a river which flows past the packinghouse. A survey of records revealed that between the years 1940 and 1957 the slaughter had increased from 500 tons to 1350 tons per day. The increase in waste production and biological load of the sewage which accompanied the expansion and growth of slaughtering operations is illustrated in Table 1.

The increase in waste production, during these years, resulted in shorter detention periods and increased flow velocities so that efficiency for removal

TABLE 1

Increase in Waste in Seventeen Year Period

	Total-Solids Tons/Day	Grease Tons/Day	B.O.D. Tons/Day	Volume M.G.D.	Slaughter Tons/Day
1940	24.3	1.8	33.6	1.4	500
1957	115.4	27.8	56	8.8	1350

of solids, fat and other organic compounds contributing to the 5-day BOD in the plant interceptor settling basins was drastically reduced. The stronger plant effluents required additional treatment at the municipal plant which was also experiencing operational difficulties due to population growth during the same period of time. These facts focused the attention of the packinghouse management on the possibility that studies made on plant waste production might ultimately result in a substantial savings to the company.

The first project was designed to indicate the hourly and total waste production within the plant. The sewers discharging into the interceptor basins were traced and the processes they served were studied in order to determine their contribution to the total hydraulic and biological load. Hourly samples, based on volumes of flow, were collected and analyzed in the laboratory, according to Standard Methods (6), for total, suspended, and volatile solids, ash, nitrogen, 5-day BOD, temperature, and pH. Records were also kept of the total pounds of liveweight animal slaughter, blood, and tankage production and inedible grease recovery so that the results of the survey could be interpreted in terms of waste production and by-product recovery per 1000 lbs. of liveweight kill. Data were collected for several months and provided a material balance of the plant's waste production.

The information obtained from this study showed clearly that some operations result in strong wastes and others in relatively weak ones. It was found that waters originating in the inedible rendering departments and sludge from the interceptor basin bottoms were major contributors to the high fat and BOD content in the plant sewage (Fig. 1). This led to the observation that these stronger wastes might merit segregation and separate treatment.

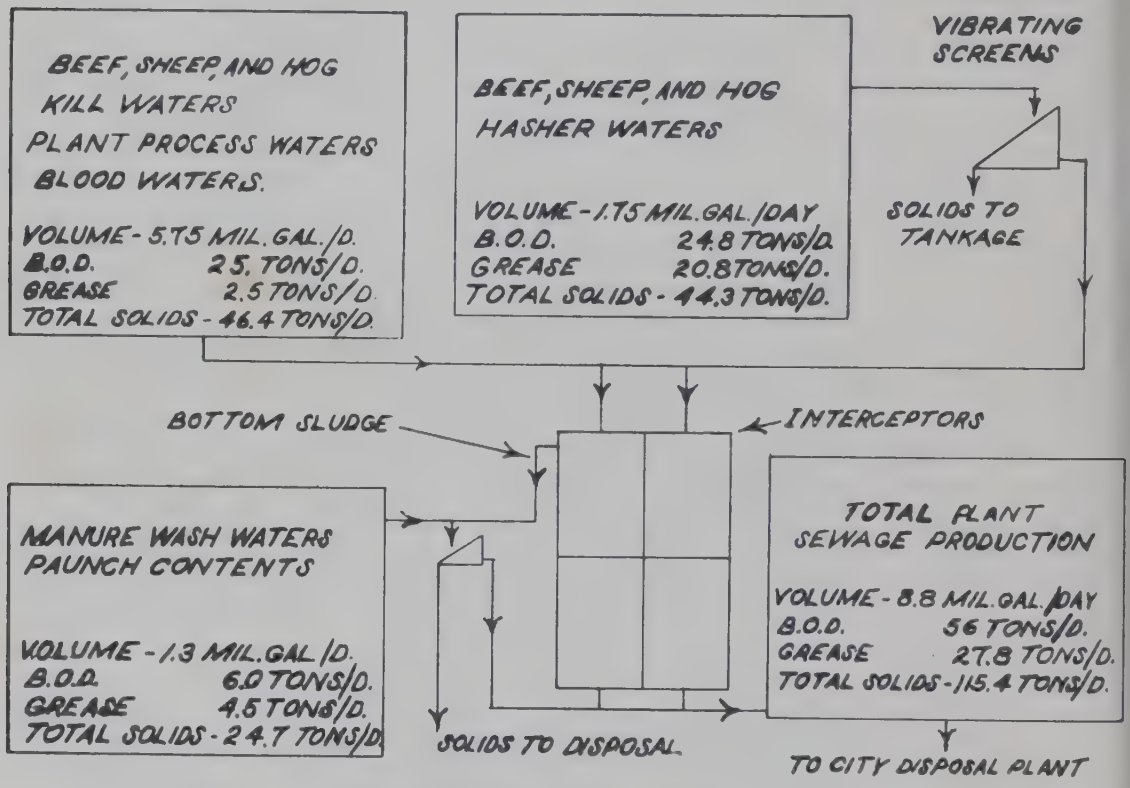

Figure 1. Flow diagram—waste treatment processes.

The plant under study employs both wet and dry rendering processes for rendering inedible animal tissues. The dry rendering process does not produce liquids other than clear condenser waters which are discharged to the river. The wet rendering process produces an intermediate product called

ank water. The larger volume of this material is evaporated into stick for incorporation into tankage.

In both processes, however, it is customary to hash and wash the tissues before rendering. The wash waters from the rotating hasher drums were analyzed and found to contain large amounts of grease, tissue particles, and manure solids. Vibrating screens removed larger than 20 mesh size particles from these waters and the screen underflow was discharged into the interceptor basins for settling and skimming.

The other process which was found to be a major contributor to the strength of the plant wastes was that of screening interceptor bottom sludge. Sludge recovery consisted of pumping bottom solids from the settling basins over vibrating screens. These screens were also employed to remove solids from paunch contents and stock pen waste waters (8). Composite samples of the underflow from the paunch and manure screens showed that the BOD of this liquid waste was between 200 and 400 p.p.m. The low BOD is due largely to the volumes of clear waters which are used for washing the paunch and cleaning the stock pens. These findings were in agreement with other investigators who have reported that the BOD of these waste waters, particularly in federally inspected plants, was relatively low (7). However, it was found that when bottom sludge was pumped over these screens, particles fine enough to pass through practical mesh screens were the major constituent of the suspended solids and BOD of the waste waters discharged to the municipal disposal plant.

With these facts established, it became desirable to investigate ways and means of recovering profitable materials and elimination of others from the plant sewage. The salvage processes which were applied on pilot and full plant scale and the modifications in waste handling which have resulted from this study are the subjects of this paper. Data are presented for each application and discussed as to which processes recover specific materials most economically.

EXPERIMENTAL

An attempt was made to reduce the suspended solids, grease and BOD of the hasher wash waters and manure screen waters by employing air-flotation. Air-flotation is a method of treatment whereby air is forced into the liquid waste during its passage through a small pressure tank. As the air pressure is slowly released, air bubbles rise and carry grease and suspended matter to the surface where they are removed by mechanical skimmers (Fig. 2). The denser solids settle to the bottom and are removed as sludge. Various coaguants were employed to increase the effectiveness of the solids separation.

The next study concerned the separation of solids in a liquid suspension by employing the principles of classification. The objective of this work was to separate the heavier solids from the light fat particles in the screened hasher wash waters.

The basic theory of classification has been described as the maximum settling velocity of a solid particle (2). When there is sufficient difference in the specific gravities and size of particles in a suspension and if the terminal velocities are great enough, classification may be accomplished by using the force of gravity. A simplified schematic diagram of a pilot plant cyclone is depicted in Figure 3. This type of cyclone was constructed in our mechanical shops. The unit consisted of a cylinder, five inches in diameter, mounted on a conical base. Feed entered tangentially at the cylindrical section. The bottom outlet is at the base of the cone. The tangential flow results in a double-spiral vortex. The outer spiral moves toward the outside and is drawn down to the apex of the cone at the bottom. This is termed the underflow. The inner spiral

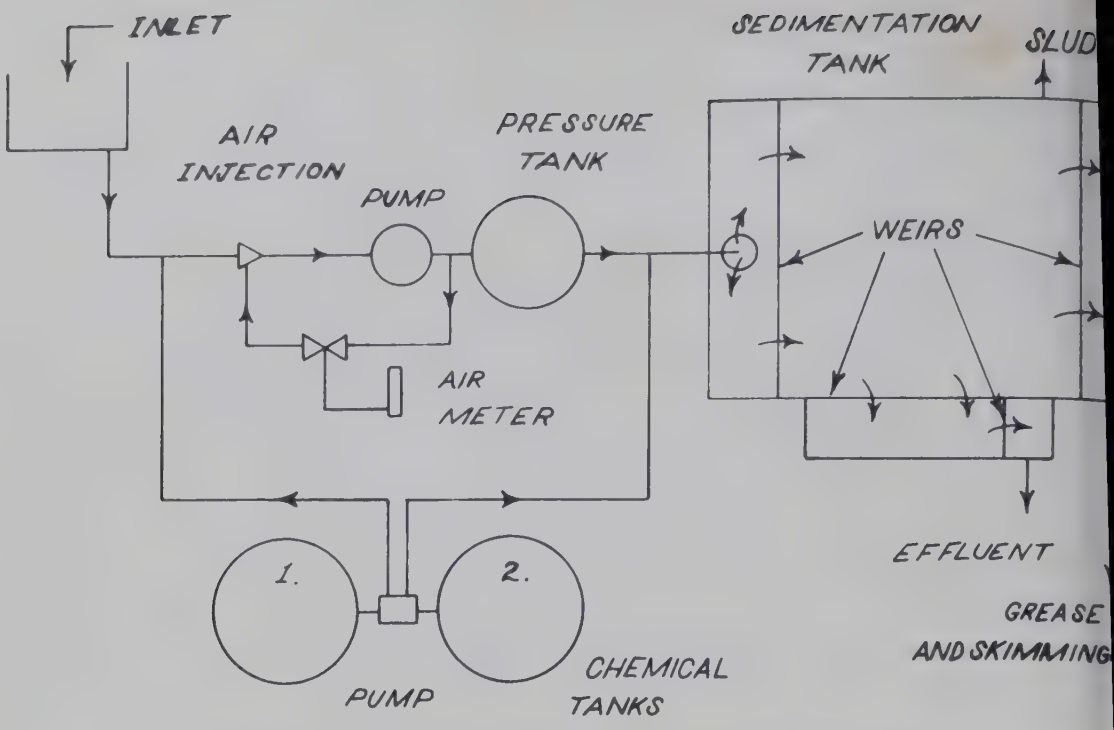

Figure 2. Flow diagram—air flotation pilot plant.

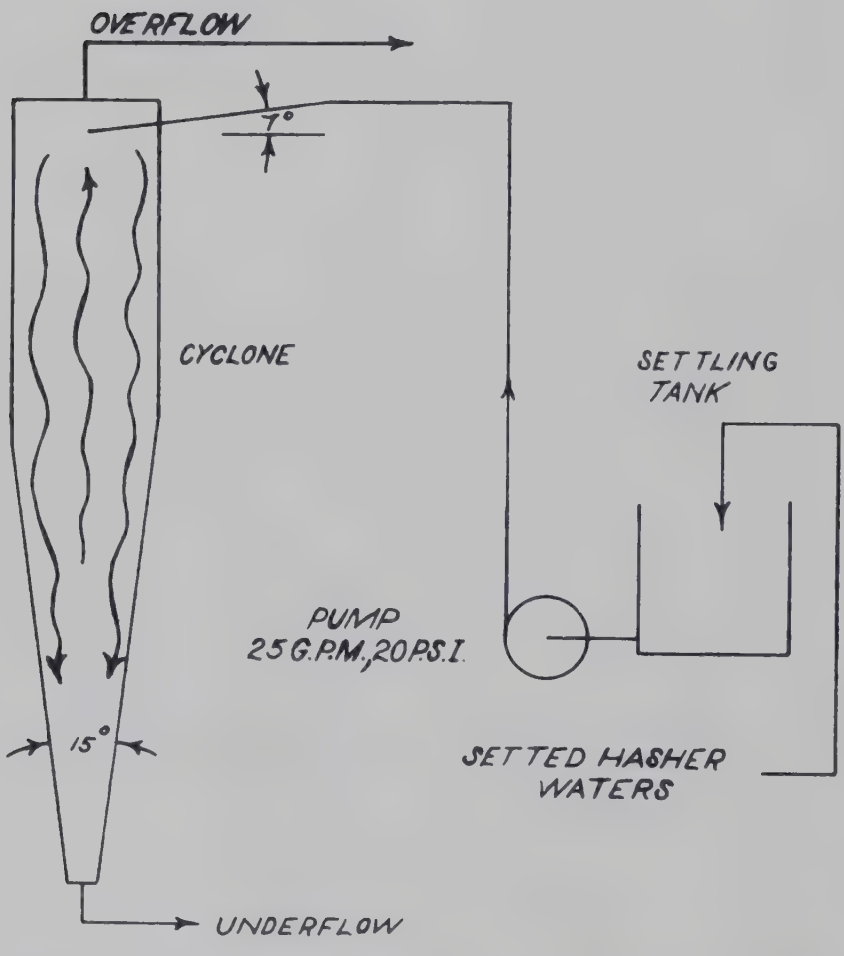

Figure 3. Flow diagram—cyclone classification of solids.

w rotates in the same direction but moves upward toward the overflow tube. avier particles are thrown toward the outside of the cone and are removed the underflow. Lighter particles move toward the center of the cone and up- rd to the top overflow. Samples of hasher wash waters containing 3900 p.p.m. spended solids and 1040 p.p.m. grease were fed into the cyclone. A pressure 20 psig. was maintained. Analyses were made of the overflow and underflow m cyclone.

In order to study the application of vacuum filtration as a means for re- vering interceptor bottom sludge, the Büchner funnel method as modified by ck, Sakellarious and Krup was employed (1). In addition, a vacuum filter lf, measuring 0.1 sq. ft., was obtained (Dorr-Oliver, Inc., Stamford, Conn.). mples of bottom sludge were forced through various types of filter media. fferential pressures were maintained across the medium and the insoluble lids were collected on the surface of the filter leaf. These preliminary tests dicated that the process would be effective for recovering solids from a dge slurry. It was also found that addition of a filtering aid, such as Grade eed Plus, Dicalite (Great Lakes Carbon Corporation, New York, N. Y.) im- oved filtration efficiency. A pilot-plant size, pre-coat type of vacuum filter th a 50 gpm. capacity was obtained from Dorr-Oliver, Incorporated. This pe of filter consists of a drum which rotates about a horizontal axis. A por- on of the drum is submerged in the slurry which is to be filtered (Fig. 4).

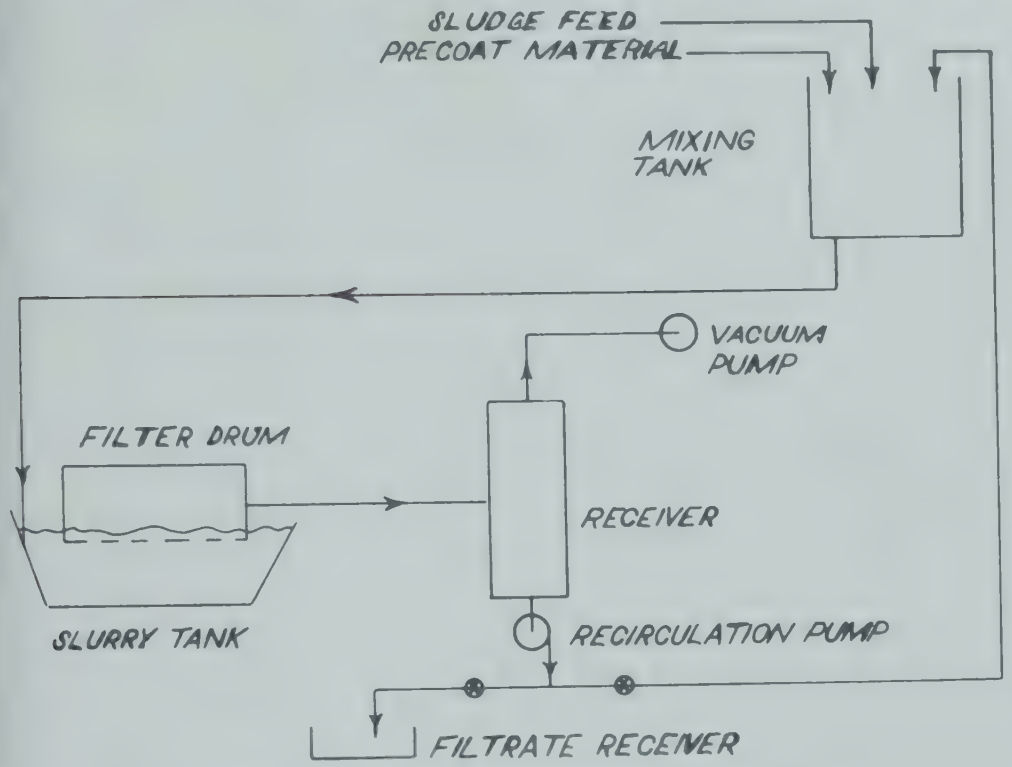

Figure 4. Flow diagram—vacuum drum filter.

he interior of the drum is divided into a number of longitudinal sections hich are connected to a common stationary valve. The outside of the drum s constructed of a perforated stainless steel plate which is covered by a po- ous filter medium. The filter valve is attached to a vacuum system so that ne drum sections can be subjected to a negative pressure on the inner side of he filter medium. As the drum rotates through the slurry, the liquid is drawn hrough the cloth by the vacuum on the inner surface of the drum. The solids n the liquid form a filter cake on the outside of the cloth and a scraper re- loves a pre-determined amount of the cake before the drum submerges for

the next revolution. Samples of interceptor sludge were concentrated by s tling and fed to the filter at various rates. Analysis of the filter effluent w obtained as well as the amount of cake which accumulated.

To determine the possibility of separating solids from a slurry by me of a centrifuge, samples of sludge were concentrated to 1% and 3.5% total ids by settling. Representative portions of both samples were gravity fed a various rates to a Sharples Super-D-Canter (The Sharples Corporation, Pi delphia 40, Pa.). The unit was operated at 6000 RPM in order to simulate plant size centrifuge.

The preliminary studies indicated the possibility of segregation and se arate treatment of high strength waters to improve grease recovery and re duce solids content. The plant methods of processing these materials were modified in the following manner:

1. The hasher wash waters were segregated in one primary basin without screening.
2. Settled effluent from the secondary basins was recirculated for re-use in the hasher sprays.
3. Sludge from the primary basins was screened over the vibrating screens which had previously been employed for separating solids from hasher waters.
4. The screen underflow was recirculated to the primary basins for additional settling.

RESULTS AND DISCUSSIONS

Air Flotation. It was established that a definite reduction of fat, solids, and 5-day BOD could be obtained by employing the air-flotation process on hasher wash waters and manure screen waters (Table 2). It was also found that the addition of 300 p.p.m. aluminum sulphate accelerated flocculation a improved the reduction in the strength of the wastes. Further investigation revealed, however, that the process resulted in decreased volumes and qual ties of recoverable grease. The grease which was reclaimed by the process was a poor grade, number two tallow and dark in color. Cost of chemicals f treating 1,000,000 gallons of these waters per day was $134.00. Capital inve ment for equipment approximated $30,000.00.

Liquid-Solid Cyclone. It was found that under controlled conditions this process was effective for separating solids from concentrated waste waters It appeared that 50% of the suspended solids could be concentrated into 15% the total volume of waters treated (Table 3). The underflow from the cyclon contained a large amount of fat-free solids while the overflow contained a large volume of relatively clear effluent. It was observed, however, that for consistent results on a plant-scale basis, higher pressures and larger volumes of flow were required than were possible with the existing equipment.

Vacuum-Drum Filtration. It was demonstrated that vacuum drum filtra tion reduced the solids content in bottom sludge by 80%. Total solids in the sludge slurry at the feed inlet of the filter were 5548 p.p.m. At a feed rate 6.26 gph. per sq. ft. of filter surface and a vacuum of 20 inches, an effluent could be produced with a total solids content of 1090 p.p.m. (Table 4). The fi ter cake accumulation was at the rate of 0.38 lbs. per sq. ft. of filter surfac area. The cake had a moisture and ash content of 51 and 46%, respectively. These results indicated that the rate of filtration was not as high as was expected. For the desired application, three 100 sq. ft. filters would be requir at an estimated capital investment of $100,000.00. In addition, facilities wou be required for disposal of the filter cake.

Centrifugation. It was found, on the basis of the laboratory tests, that

TABLE 2

Analytical Results—Air Flotation

Settled Sludge from Hasher Waters
Volume 1,750,000 GPD

	Inlet ppm	Outlet ppm	Reduction %
5-day BOD	1614	237	85
Grease	1944	142	93
Sus. solids	2657	495	81
Tot. solids	4835	1904	61

Manure Screen Underflow
Volume 1,300,000 GPD

	Inlet ppm	Outlet ppm	Reduction %
5-day BOD	936	224	76
Grease	814	37	97
Sus. solids	2032	183	91
Tot. solids	4150	1311	68

TABLE 3

Classification of Solids By Cyclone

	Average Results		
	Feed 100%	Underflow 15%	Overflow 85%
	Lbs./1000 Lbs.	Lbs./150 Lbs.	Lbs./850 Lbs.
Sus. Solids	3.9	2.3 - 59%	1.6 - 41%
Grease	1.04	.32 - 31%	.72 - 69%
Protein	.72	.38 - 50%	.38 - 50%
Ash	.57	.45 - 79%	.12 - 21%
Fiber	1.57	1.15 - 73%	.38 - 27%

entrifugation would remove 70-75% of the solids, by volume, in bottom ludge at the rate of 15,000 gph. (Table 5). The clarified mother liquor from he heavy and light settled sludge contained 0.8% and 0.3% solids, by volume, espectively. The recovered solids contained 55-60% moisture. These re- ults indicated that, for application at this plant, three centrifuges would be equired at a total cost of approximately $140,000,00 for a feed of 45,000 gph. acilities for disposal of the solids from the centrifuge would also be neces- ary.

TABLE 4

Solids Reduction by Vacuum-drum Filtration

Settled Sludge Inlet to Filter	Flow Rate Gal./Hr./Sq.Ft.	Slurry Tot. Solids	Filtrate Tot. Solids	Filter Cake	
				Recovery Lbs./Hr./Sq.Ft.	H_2O %
Test		ppm	ppm		
A	6.26	5,548	1,090	.378	51
B	5.93	5,580	1,332	.93	52
C	5.56	8,309	6,033	.15	44
D	6.60	7,210	2,462	-	-
E	1.68	5,330	2,876	-	-

TABLE 5

Recovery of Sludge Solids by Centrifugation

Sludge Feed % Solids Concentration	Effluent Rate GPH	Solids in Effluent % by Volume	Recovery of Solids from Sludge* %
3.5	2000	0.6	83
3.5	3300	0.8	77
3.5	5000	0.8	77
1.0	2000	0.2	80
1.0	3000	0.2	80
1.0	5000	0.3	70

*Moisture content of recovered solids: 55-60% at 105°C.

Modification of Plant Operations

1. Segregation of Hasher Wash Waters. Laboratory analyses of the hasher water screen underflow revealed a grease content of 2850 p.p.m. Measurement of the flow volume from the hashers resulted in a figure of 1.7 mgd. This data indicated that these waters contained approximately 40,000 lbs. of a choice grade of grease daily. It was further shown that recovery of this grease became more difficult when these waters were mixed with approximately 5.75 mgd. of plant flow in the interceptor basins. These results and observations led to the segregation of the hasher waters in one primary basin. This provided a one hour and ten minute detention time for these waters and resulted in a 25% increase in grease recovery. It was also observed that the large volume of flow from the hasher drums required five 4' x 7' vibrating screens. It was also noticed that the high grease content of these waters caused "blinding" and that two men were required to wash and keep the screens clear. These observations led to the elimination of the screening process so that the wash waters from the rotating hasher drums were discharged directly into the primary basin for settling and skimming. An improved separation of the grease from the solids was experienced and grease recovery by skimming increased 6000 to 8000 lbs. per day. The monetary value of this grease was assessed at 6¢/lb. and it was shown that these modifications resulted in a saving of approximately $450.00 per day.

2. Recirculation of Settled Waters to Hasher Sprays. It was the practice

use fresh, clear water to wash the interior of the rotating hasher drums. xperimentation proved that settled waters from the secondary settling ba- ns were of sufficient clarity to recirculate and use for the hasher sprays. e-use of these waters reduced the hydraulic load by approximately one mil- on gallons per day.

3. Screening Sludge over Vibrating Screens. It was found that after the nscreened hasher waters were segregated in the primary basin, the bottom olids could be recovered by employing the vibrating screens. The increased etention time for the hasher wash waters resulted in improved settling and oncentration of the solids. Laboratory analysis revealed that the solids were roteinaceous in nature, containing 28.46% protein and 25.49% fat on a dry eight basis.

The relatively lower volume of highly concentrated solids, after settling, equired less screen capacity and since the floatable grease had been removed om these solids it was found that "blinding" of the screens did not occur. hen the settled sludge was pumped over the 20 mesh screens a blanket was ormed which increased the filtration efficiency. It was also established that ne recovered solids were of sufficient feed grade quality for wet-rendering nto tankage.

4. Recirculation of the Screen Underflow. After it was established that ne bottom solids could be recovered by employing the vibrating screens, our ttention was directed to the treatment of the screen underflow. Laboratory nalysis indicated that these waters contained soluble protein, grease, par- icles of less than 20 mesh size, enzymes and bacteria. An attempt was made o improve the quality of this underflow by recirculation to the interceptor asins for additional settling. It was discovered that this modification result- d in a marked increase in the volumes of grease recovered by the interce p- or basin skimming operation.

In order to study the effects of recirculating the screen underflow, set- ling and rise rate tests were performed. The rise rates of the grease and the settling rates of the solids were measured by means of a graduated 1 liter cyl- nder and a stop watch. It was observed that the maximum volume of settle- able solids in the fresh hasher influent to the interceptor settling basins was approximately 15 ml. in 30 minutes. It was found that when the screen under- low was recirculated to mix with the raw influent to these basins, the settle- able solids increased to 300 ml. in 30 minutes. It was also observed that a more clearly defined separation of solids and grease was obtained in the liter cylinder. These findings were then confirmed on full plant scale tests. The av- erage amount of grease recovered by skimming the interceptor basins without recirculation of the screen underflow was approximately 7.5 lbs. per ton of slaughter. With the screen underflow mixed with the raw influent, the grease recovery increased to approximately 14 lbs. per ton of slaughter.

While the mechanics of this reaction are not clearly understood, it is be- lieved that the inoculation of the raw waste with the pre-incubated, seeded un- derflow, followed by a short incubation period in the main settling basins, pro- duces symbiotic changes which accelerate the separation of solids and grease. The temperature of the waters in the interceptor settling basins is approxi- mately 95° F. and it is believed that these temperatures aid in the incubation of the seeded raw wastes so that a crude aerobic digestion system is achieved. These modifications of in-plant waste handling methods have resulted in up to 50% increases in grease recovery from the interceptor skimming operation and have also reduced the strength and character of the waste waters leaving the plant.

A comparison of the amount of materials lost in the sewage, per 1000 lbs. of live-weight slaughter, for the year 1955 and the year 1958 is shown in Ta- ble 6. It should be noted that the volume of waste production has been reduced from 8.8 mgd. to 6.6 mgd.; grease losses have been reduced from 27.2 lbs. to

7.6 lbs.; suspended solids show a reduction from 35.5 lbs. to 7.6 lbs.; and th 5-day BOD of the plant sewage has been reduced from 40.3 lbs. to 25.4 lbs.

TABLE 6

Comparison of Waste Production, 1955 vs. 1958

Before and After In-plant Modifications	1955	1958
Total waste production (MGD)	8.8	6.6
Liquid waste per 1000 lbs. kill (gal.)	3500	2234
Suspended solids per 1000 lbs. kill (lbs.)	35.5	7.6
Grease per 1000 lbs. kill (lbs.)	27.2	7.6
BOD production per 1000 lbs. kill (lbs.)	40.3	25.4

SUMMARY

A study of various types of waste-handling equipment was made and included processes such as air-flotation, liquid-solid cyclone classification, vacuum-drum filtration, and centrifugation. It was found that each process could be applied for reducing the biological load and solids content in packinghouse wastes. However, for the plant under study, the high capital investment, operating costs or low rates of material recovery precluded their application. The most effective methods for reducing valuable material losses were determined by studying each plant waste handling process separately and evaluating its contribution to the total hydraulic and biological load. Modifying the methods of handling hasher wash waters and interceptor bottom sludge resulted in increased grease and green tankage recovery as well as reduced hydraulic and biological load of the sewage. In the course of our investigation, it was observed that the effect of seeding the raw sewage may play an important role in material recovery and purification of waters polluted with grease and proteinaceous solids. A further study of the system described may reveal important physical and chemical changes and inter-relationships taking place when the pre-incubated, seeded underflow from the sludge screens is brought into contact with fresh packinghouse sewage.

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XI

THE RELATIVE SIGNIFICANCE OF FACTORS INFLUENCING AND/OR ASSOCIATED WITH BEEF TENDERNESS*

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Of the palatability attributes of beef, tenderness is one of the most important. The meat industry has shown marked interest in the problem of beef tenderness; research in this area has increased very materially during the last few years. The validity or usefulness of two basic concepts of carcass merit has been challenged—the textbook principle that marbling contributes to tenderness and the broad idea that carcass maturity or age of the animal or time of slaughter influences tenderness.

Factors such as finish, animal age at time of slaughter and breeding have been studied, singly, to provide a better understanding of beef tenderness. Numerous studies have centered around the relationship between fatness or finish and tenderness. Black et al. (1) and Branaman, Hankins and Alexander (2) in earlier studies found little or no relationship between finish and tenderness. In 1956, Cover, Butler and Cartwright (5) reported a positive association between fatness and tenderness but the relationship was not high. With broiled loin steaks, only 10 per cent of the variability in tenderness was accounted for by variations in fatness. Inasmuch as marbling of the ribeye is indicative of fat dispersion within lean tissue, other workers have studied the marbling - tenderness relationship. Stouffer and Wellington (9) found marbling to be only moderately associated with tenderness of steaks. A similar finding was reported by Palmer et al. (8) who noted that marbling explained only 8 per cent of the variability in tenderness as evaluated by taste panel.

Two studies have indicated tenderness differences among certain breeds and lines of cattle. In 1955, Carpenter et al. (3) reported that as the percentage of Brahman breeding increased, the tenderness of steaks and roasts decreased. Cartwright (4) found more variability in tenderness among Brahman sire groups sampled than among the Hereford sire groups.

Beef tenderness has long been thought to be influenced by animal age at time of slaughter. Hiner and Hankins (6) studied the effect of animal age at time of slaughter. Fifty-two animals were used which varied widely as to age; the study was summarized to the effect that as animal age at time of kill increased, tenderness decreased. Similar conclusions have been reported in other studies involving the age and tenderness relationship. However, Palmer et al. (8) in a study involving 502 animals ranging from 5 to 30 months of age found, on a within degree of marbling basis, a slight but insignificant positive relationship between age at slaughter and tenderness. Disregarding degree of marbling, a highly significant positive correlation of .15 was obtained. Thus,

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among these carcasses, an increase in age per se was not associated with decrease in tenderness.

Although singular effects of finish and/or marbling, age at time of sla ter and breeding are reported in the literature, little was known of the rela tive significance of the major factors influencing and/or associated with be tenderness.

The purpose of this investigation was to study the relative effects of gr marbling, age at time of slaughter and breeding on beef tenderness.

EXPERIMENTAL

Study 1

The 281 animals used in this study were raised at the Range Cattle Sta tion, Ona, Florida. Breeding was predominantly Brahman and Shorthorn; ex perimental animals were sired by 8 Brahman, 6 Shorthorn and 2 crossbred bulls. Ages at time of slaughter ranged from 5 to 87 months with an average of 18.8 months. Forty-eight hours following slaughter, carcasses were grad to the nearest one-third of a grade by a USDA federal meat grader. Carcass grades ranged from low canner to low prime and averaged between high sta ard and low good. Marbling scores given by the federal grader ranged from devoid to moderately abundant and averaged between traces and slight amou of marbling. A six inch section of short loin was removed from the left side of the carcass, forty-eight hours after slaughter, and immediately wrapped for freezer storage, frozen and held at 0° F. until tests were made. Steaks one inch in thickness were cut from short loin sections and thawed at 36° F. for twenty-four hours. Adjacent steaks for panel and shear tenderness evalu ation were broiled simultaneously in electric ovens to a uniform "medium" degree of doneness. The Longissimus dorsi muscle was removed from the broiled steak and trimmed of exterior fat and connective tissue. The muscle was cut perpendicular to its long axis into four samples and served to a four member tenderness panel. To avoid possible variations in tenderness within the muscle, a panel member consistently received samples from the same area of the steak. Three one-half inch diameter cores were obtained along a line at midpoint of the muscle and parallel to its axis. Each core was sheare twice, using the Warner-Bratzler shear apparatus. Through replication, aver ages of eight taste panel and twelve Warner-Bratzler shear evaluations were obtained for each carcass.

Estimates of heritability were derived by the method of intraclass corre lation of offspring of sires of the same breed. Covariance analysis was used to adjust for age at slaughter in mean square errors of estimate.

Study 2

To further evaluate the tenderness effects of age of animal at time of slaughter, marbling and carcass grade, disregarding breed and sire, tender ness evaluations of 180 of the carcasses used in Study 1 were combined with 322 carcasses of undetermined genetic background to provide a total of 502 carcasses for Study 2. Carcass chilling, sampling and steak broiling method of Study 2 were similar to methods employed in Study 1. All carcasses were from animals ranging in age at time of slaughter from 5 to 30 months.

Simple correlation coefficients between tenderness values obtained by panel and age at time of slaughter for each marbling group were calculated.

RESULTS AND DISCUSSION

udy 1

The gross effects of breed of sire and of sire within breed are shown in able 1 by use of average panel tenderness values. It may be noted that the erage observed panel tenderness scores for sires vary markedly. Among ahman sires, average values ranged from 4.45 to 3.24 while among Short- rn sires average values varied from 4.36 to 3.95. The means for crossbred res may lack being representative due to small numbers of progeny sam- ed. As shown in Table 1, adjustments for age at time of slaughter alter sire eans only slightly; however, on an adjusted mean basis two Brahman sires ere most tender of all sire groups and three were above average in tender- ss. The frequency distribution of panel tenderness scores for Brahman and r Shorthorn progeny is slightly skewed as seen in Figure 1. Other calcula- ons indicate that sixty per cent of the Brahman progeny were less than aver- e in tenderness, whereas sixty-one per cent of the Shorthorn progeny were erage or above.

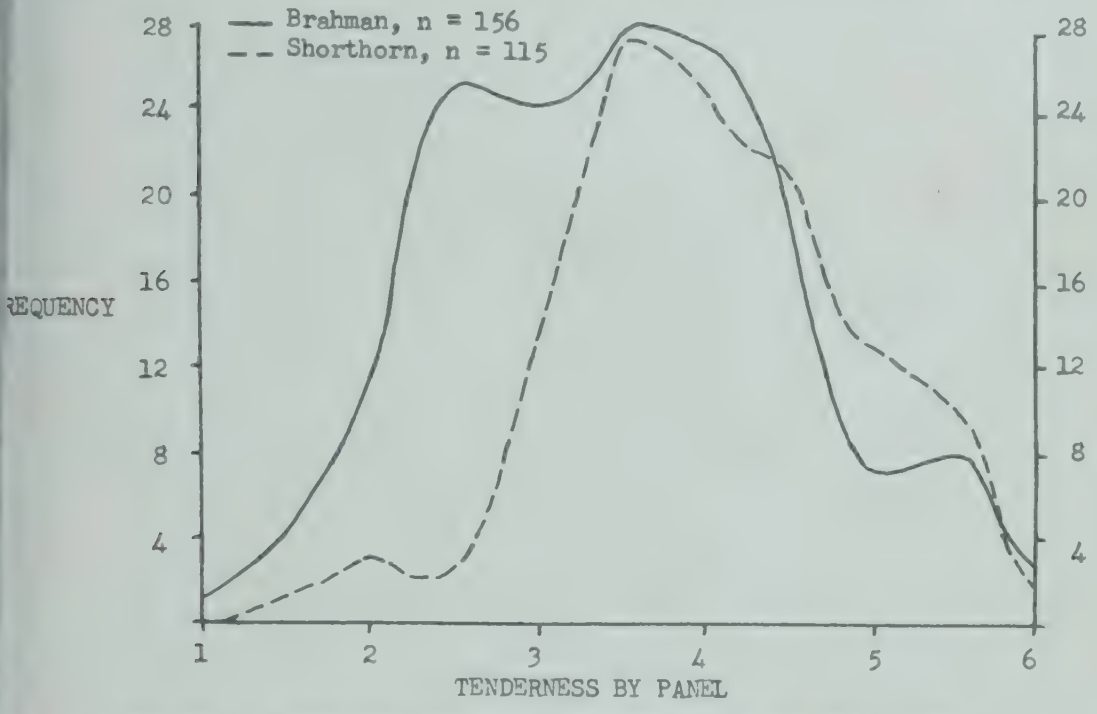

Figure 1. Distribution of progeny by breed for tenderness by panel. Panel tenderness scale – 6, excellent; 5, above average; 4, average tenderness; 3, slightly tough; 2, extremely tough; 1, inedible.

Differences due to sires, breed of sire and sires within breed were all ighly significant for both tenderness measures (Table 2). Although tender- ess differences among Shorthorn sires were insignificant, differences among Brahman sires were highly significant for both panel and shear values (Ta- le 3).

Heritability estimates measure the extent to which differences in hered- y influence tenderness differences between animals. The heritability esti- nates (Table 4) derived from Brahman sires were 71 and 54 per cent for pan- l and shear tenderness values, respectively. Heritability estimates (Table 4) or Shorthorn sires were zero for both tenderness measures. Shorthorn sires sed in this study were quite similar genetically and thus hereditary differ- nces in tenderness should not be expected to be pronounced. The genetic sim-

TABLE 1

Observed and Adjusted* Panel Tenderness Scores† by Sires and Breeds

Brahman				Shorthorn				Crossbred			
Sire	No. Progeny	Observed	Adjusted	Sire	No. Progeny	Observed	Adjusted	Sire	No. Progeny	Observed	Adjusted
1	5	4.45	4.50	1	9	4.36	4.30	1	6	3.52	3.51
2	6	4.25	4.38	2	23	4.28	4.23	2	4	2.60	2.58
3	25	4.21	4.21	3	35	4.11	4.16				
4	15	3.59	3.56	4	29	4.08	4.07				
5	29	3.50	3.53	5	10	3.99	3.97				
6	24	3.41	3.39	6	9	3.95	3.98				
7	15	3.37	3.29								
8	37	3.24	3.27								
Breed Mean		3.59	3.59			4.13	4.11			3.15	3.14

*Adjusted for age at slaughter.

†Panel tenderness scale - 6, excellent; 5, above average; 4, average tenderness; 3, slightly tough; 2, extremely tough; 1, inedible.

TABLE 2

Mean Square Errors of Estimate from Covariance Analysis of Age at Slaughter (x_2) and Tenderness Score by Panel (y_1) and Warner - Bratzler Shear (y_2)

Source	df	Tenderness	
		Panel Mean Square	Shear Mean Square
Total	279	.92	8.94
Within sire	264	.79	8.09
Sires	15	3.25**	23.96**
Breed of sires	2	10.15**	48.75**
Sires within breed	13	2.19**	20.14**

**Significant at .01 level.

TABLE 3

Analysis of Variance for Panel Tenderness and Shear Tenderness by Breed of Sire

Source	df	Mean Square	
		Panel	Shear
Brahman Sires			
Total	154		
Within sires	147	0.854	9.230
Brahman sires	7	3.176**	27.914**
Shorthorn Sires			
Total	113		
Within sires	108	0.670	6.778
Shorthorn sires	5	0.196	4.214

**Significant at .01 level.

TABLE 4

Heritability Estimates for Tenderness by Panel and Shear Based on Intraclass Correlation of Half Sibs

Sires Used in Estimate	Heritability	
	Panel	Shear
Brahman (8 sires—156 progeny)	72	54
Shorthorn (6 sires—115 progeny)	0	0
Pooled, sires within breed (16 sires—281 progeny)	57	49

TABLE 5

Simple, Partial and Multiple Correlations of Grade (x_1), Slaughter Age (x_2) and Marbling (x_3) with Tenderness by Panel (y_1)

Source of Variation	Coefficients of Correlation			Per Cent Variability Accounted for		
	All Progeny	Brahman[1]	Shorthorn[2]	All Progeny	Brahman[1]	Shorthorn[2]
Correlation						
yx_2 age effect	-.28**	-.18*	-.34**	7.7	3.1	11.5
yx_3 marbling effect	.26**	.31**	.20**	6.6	9.7	4.0
$yx_2.x_3$ age effect (marbling constant)	-.28**	-.26**	-.39**	8.0	6.7	11.4
$yx_3.x_2$ marbling effect (age constant)	.26**	.29**	.20*	6.9	8.6	3.9
$R.x_2x_3$ (marbling and age constant)[3]	.38**	.40**	.39**	14.1	15.7	15.0
yx_1 grade effect	-.34**	-.37**	-.28**	11.5	13.9	7.8

[1] Brahman progeny.

[2] Shorthorn progeny.

[3] Multiple correlation based on partial regressions of age and marbling to show the combined effects of marbling and age on observed tenderness.

* Significant at .05 level.

** Significant at .01 level.

close line breeding. Kieffer et al. (7) recently reported differences in tenderness among Angus sire groups with an estimate of heritability of 92 per cent for tenderness by shear. Differences in tenderness and relatively high estimates of heritability among Brahman sires suggests the possibility for progress through breeding for tenderness.

To study the relationships of age at slaughter, marbling and carcass grade to tenderness as influenced by breed of sire, simple, partial and multiple correlation coefficients were computed on a within sire basis for all progeny, Brahman progeny and Shorthorn progeny. Data are presented in Table 5. With marbling held constant, the effect of slaughter age on panel tenderness was more pronounced among Shorthorn than among Brahman progeny with highly significant coefficients of -.39 and -.26, respectively. Among all progeny, slaughter age, with marbling constant, had only a slightly stronger influence on panel tenderness than did marbling with age constant.

When age was held constant, marbling influenced the tenderness of Brahman progeny more than Shorthorn progeny. Holding age constant, a highly significant partial correlation coefficient of .29 was found between marbling and panel tenderness for Brahman progeny whereas the corresponding coefficient for Shorthorn progeny was a significant .20. Holding age constant, marbling among Shorthorn progeny had only a slight relationship with panel tenderness and accounted for 3.9 per cent of panel tenderness variability.

The multiple correlation coefficient is a measure of the combined effects of two independent variables on a dependent variable. Since marbling and age are the main factors determining carcass grade, multiple correlations of tenderness score with marbling and age could be compared to simple correlations between grade and tenderness. Among Shorthorn progeny, a multiple correlation coefficient of .39 was obtained whereas the simple correlation between carcass grade and panel tenderness was -.28, indicating that age and marbling affected tenderness slightly more than did carcass grade, the negative sign in the grade correlation being due to coding used in analysis. Marbling and slaughter age showed a slightly greater relationship to panel tenderness than did carcass grade among all progeny, regardless of breed of sire.

As shown in Table 6, age at slaughter, with marbling held constant, was not significantly correlated with tenderness by shear among Brahman progeny; however among Shorthorn progeny a highly significant partial correlation coefficient of .35 was obtained when marbling was held constant. A partial correlation coefficient of .25 was found between slaughter age and tenderness by shear among all progeny, with marbling held constant, whereas a partial correlation coefficient of -.35 was obtained between marbling and tenderness shear when age was held constant. Thus marbling influenced shear tenderness more strongly than did age at slaughter. With age held constant, marbling showed a stronger relationship with tenderness shear among Brahman progeny than among Shorthorn progeny. The combined effects of marbling and age on tenderness by shear among Brahman progeny was more pronounced than the effect of carcass grade which did not correlate significantly with this measure of tenderness. Among all progeny and Shorthorn progeny, carcass grade correlated equally as well with tenderness by shear as did the combined effects of marbling and age.

Preliminary data as to the relative significances of factors influencing or associated with beef tenderness are presented in Table 7. Breed of sire and the sires within breed were the two factors accounting for the highest percentage of variability in tenderness; breed of sire accounted for 13.6 per cent and sires within breed 14.3 per cent. Carcass grade accounted for 11.5 per cent of the variability in tenderness. In this study, Shorthorn progeny outgraded the Brahman progeny. Hence some tenderness variability attributed to carcass grade could be sire effect; further, certain variability attributed to breed of

TABLE 6

Simple, Partial and Multiple Correlations of Grade (x_1), Slaughter Age (x_2) and Marbling (x_3) with Tenderness by Warner-Bratzler Shear Technique (y_2)

Source of Variation	Coefficients of Correlation			Per Cent Variability Accounted for		
	All Progeny	Brahman[1]	Shorthorn[2]	All Progeny	Brahman[1]	Shorthorn[2]
Correlation						
yx_2 age effect	.24**	.17	.35**	5.8	2.8	12.2
yx_3 marbling effect	-.35**	-.43**	-.26**	12.0	18.8	6.9
$yx_2.x_3$ age effect (marbling constant)	.25**	.06	.35**	6.3	0.0	12.3
$yx_3.x_2$ marbling effect (age constant)	-.35**	-.41	-.27**	12.4	16.8	7.1
$R.x_2x_3$ (marbling and age constant)	.42**	.20	.43**	17.5	4.0	18.4
yx_1 grade effect	.45**	.12	.43**	20.2	1.3	18.6

[1]Brahman progeny.

[2]Shorthorn progeny.

[3]Multiple correlation based on partial regressions of age and marbling to show the combined effects of marbling and age on observed tenderness.

*Significant at .05 level.

**Significant at .01 level.

ire and sires within breed could be carcass grade effect. Age at time of laughter and marbling accounted for 8.1 and 6.9 per cent of the variability n tenderness. Variability of panel tenderness accounted for by breed of sire nd sires within breeds suggests that these factors outweigh increases in enderness gained through increased marbling and youthfulness.

TABLE 7

Per Cent of Tenderness Variability Accounted for by the Factors Studied—All Progeny

Factor	Panel Tenderness Per Cent	Shear Tenderness Per Cent
Breed of sire	13.6	5.4
Sires within breed	14.3	12.2
Carcass grade	11.5	20.2
Age at slaughter	8.1	6.3
Marbling	6.9	12.4

tudy 2

The relationship of beef tenderness and age of animal at time of slaughter is presented in Table 8. Within degrees of marbling, devoid, slight, small nd modest, positive but insignificant relationships were obtained between enderness and age of animal at slaughter. A positive significant correlation oefficient of .23 was found between tenderness and age at slaughter within arcasses practically devoid in marbling. A negative but insignificant relationship was found between tenderness and age within carcasses with traces of marbling. Although the relationships lacked significance and magnitude, the positive signs indicate that within carcasses from animals 5 to 30 months of age tenderness increased slightly with age. A highly significant correlation coefficient of .15 was obtained between tenderness and age at slaughter using he 502 animals without respect to degree of marbling.

TABLE 8

The Relationship of Beef Tenderness by Taste Panel and Age of Animal at Time of Slaughter

Degree of Marbling	No. Animals	Coefficients of Correlation
		r
Devoid	46	.25 n.s.
Practically devoid	112	.23*
Traces	129	- .06 n.s.
Slight	129	.07 n.s.
Small	64	.12 n.s.
Modest	22	.03 n.s.
All degrees of marbling	502	.15**

n.s. - Not significant.

*Significant at .05 level.

**Significant at .01 level.

Using data from the above animals, simple correlations between mar bling and federal grade for both tenderness measures were highly signifi and yet, variability of grade explained only 5.6 per cent of panel tenderne variability whereas marbling accounted for 8.0 per cent.

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XII

THE CHEMISTRY OF MEAT FLAVOR—A REVIEW

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The word flavor conjures many ideas in the minds of many individuals. In order to have everyone agree on the meaning of the word, a definition of terms is desirable. A favorite definition of the author's is that flavor is a complex of sensations resulting from the stimulation of the senses of odor, taste, feel and sometimes vision and audition. To the chemist, however, only the senses of odor and taste are considered in his work on flavor.

Another term which needs definition is "flavor extract." Chemically, it is defined as that fraction of the food material under investigation which will give the same response to taste and odor as the original food. "Meat flavor extract" would then be that extract of meat which would have an odor and taste like meat but not necessarily the same flavor.

Most of the discussion in this paper will be centered around beef flavor extract. In the laboratory this material has been prepared by boiling beef muscle under reflux and separating and condensing the water-soluble portion. Such a preparation has a pronounced "broth" odor and a "meaty" taste.

Historically, beef flavor extract has had a colorful career. It has variously been treated as a medicinal with mysterious therapeutic and nutritional qualities, consigned to the kitchen as a condiment, and summarily compared to a pot of evaporated urine. Something might be said of all of these statements. Beef flavor extract has been shown to promote a feeling of well-being in the individual who partakes of it. It does contain, in small amounts, some of the vitamins. It will certainly improve the flavor of many food preparations. And, it does have a considerable number of chemical compounds in common with urine.

With such claims and counterclaims being made of its properties, beef flavor extract was naturally subject to early investigative efforts. Following reports which occur in the literature is similar to reading a chronology of the history of organic and biochemistry. The first substances identified in this biological complex were the inorganic cations such as sodium, potassium, calcium, magnesium and phosphorus as phosphate. Later came the organic materials creatine, creatinine, lactic acid, inosinic acid, hypoxanthine and inosine. Naturally, many compounds were claimed by early investigators which have not withstood proof of further scientific investigation. An example of such compound is the phosphocarnic acid which was the subject of considerable debate at the turn of the century.

The greatest strides to elucidate the composition of beef flavor extract have taken place in the last decade. The British team of Bender and Wood, working with a commercial flavor extract and utilizing modern techniques of electrodialysis and paper chromatography, have undertaken a systematic investigation of the material. Their results are of interest not only for the compounds which they have shown to be present, but also for the colligative categories which they have included in their analyses.

Thus, they have identified the amino acids—methyl histidine, alpha-alanine, serine, methionine, isoleucine, leucine, histidine, taurine and citrulline

as comprising a little more than two per cent of the total extract. This of self is surprising since most people consider the flavor of meat to be lar ly due to amino acids and to glutamic acid in particular. The peptides car sine, anserine and an unidentified iminazole peptide accounted for six per cent of the extract. Again, the small number of compounds in this categor somewhat surprising. The guanidines creatine and creatinine comprised a ten per cent of the materials present. The purines and pyrimidines, togeth with such miscellaneous organic compounds as carnitine, choline, urea an ammonia, accounted for an additional six per cent. The organic acids lacti glycollic and succinic make up sixteen per cent, protein material eleven p cent and inorganic salts, unspecified, twenty-nine to thirty per cent. This makes a total of eighty-two per cent of the material accounted for.

These figures are of themselves formidable. However, aside from the ganic acids and the inorganic salts, there is little here to account for the t and odor characteristic of beef flavor. Fortunately, these same investigato have taken cognizance of a separate category of coloring materials which comprises about eighteen per cent of the total beef flavor extract. It is no s cret to anyone who has tried to fractionate a beef flavor extract that the co ored material carries with it a great portion of the taste and odor constitue of the meat. It would be most heartening to report at this time that a compl and successful analysis of this fraction has taken place. However, such is n the case. It is known, however, that this fraction contains most of the arom substances from the meat and that there are many amine and phenol deriva tives. This particular fraction is the subject of considerable investigation i our laboratory.

No review of meat flavor would be complete without passing mention of work on poultry flavor. Diametrically opposed to the work done on beef, mo work on poultry, and chicken in particular, has been done on the volatile rat er than the non-volatile fractions. Bouthilet, and Pippen and his associates, have identified ammonia, hydrogen sulfide and eighteen carbonyl compounds as constituents of chicken flavor. Although the methods used in obtaining the results are reliable, it would be questionable whether all of the flavor was, and whether or not, of the volatiles present, only hydrogen sulfide and amm nia are present which contain sulfur or nitrogen.

In summary, it may be stated that much work needs to be done to eluci- date the chemical compounds responsible for meat flavor. In beef, despite it mouth-watering odor, most of the flavor compounds can be said to be non- volatile. The separation of these non-volatile compounds into easily identifie fractions is most difficult. To date, the best work on identifying the compou present has left much undone. This is particularly true of the material whic contributes the color, and incidentally the odor and taste, to beef extract. Th inorganic salts fraction, which makes up approximately one-third of the flav extract, is a major contributor to the flavor of the meat, but its composition has not been investigated.

Poultry, on the other hand, has been investigated primarily from the point of view of volatile components. Those compounds identified, as in beef would not likely account for the characteristic flavor.

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XIII

MEAT COOKERY FROM THE SCIENTIFIC VIEWPOINT*

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Meat cookery from the scientific point of view differs considerably from the art of meat cookery which is very ancient. In fact we know that prehistoric man existed only because he knew something about the art of meat cookery and also cared little about cleaning up after his meal. He may have used both moist and dry heat methods. The meat from the large muscles may have been roasted or braised on sticks. For smaller muscles he may have used moist heat methods. He may have dug a depression, lined it with stones, built a fire on the stones and, when they were hot, laid on them the meat wrapped in wet leaves, grass or moss. He learned to make pottery, but his pottery pots could not be used on the fire so he may have cooked his meat in water heated by adding hot stones. Thus the art of meat cookery is very old. The art of meat cookery today separates large beef muscles into two categories: "tender," for which dry heat methods are advised; and "less tender," for which moist heat methods are advised. Then there are the so-called "principles" of meat cookery: "high heat toughens protein," and "long slow cooking by moist heat methods is needed for less tender cuts to hydrolyze the collagen in the connective tissue and make it tender."

From the scientific point of view, we look on these almost as folk sayings. There may be a grain of truth but it must be searched for. Let's take the statement "high heat toughens protein." How high does the temperature have to be to qualify as high heat? And does the high heat refer to the temperature of the oven or cooking liquid or to the temperature inside the meat? Thus this statement is a highly ambiguous one from a scientific point of view. Then let's take the statement that we need "moist heat methods to soften connective tissue." How does moist heat applied from the outside of the meat soften connective tissue inside the meat? Raw meat contains about 70% water and some drips out as it cooks. Could more get inside than was there to start with? Thus this statement, too, is not at all clear from the scientific point of view.

As a way of getting at the possible grain of truth in these statements, we turn our attention to the media in which meat is cooked (air, steam, water, fat) each with its own rate of conducting heat. We control the temperature of the cooking medium and use thermometers or thermocouples to watch the rate at which the heat penetrates into the meat. After cooking in a controlled manner, we test the effect of each variation on the eating quality of the meat. When changes in eating quality are obtained under such controlled conditions the causes are pursued into the fields of histology, chemistry and physics in one direction and into the fields of processing, production and heredity in the other direction.

We need to consider next the kind of organoleptic tests which are appropriate or inappropriate for the scientific study of the causes of juiciness, fla-

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vor and tenderness. If our problem is a simple one we may use a test of the comparative type such as the paired eating method, or the duo-trio. In many of our studies, however, we need to make not one or two but many comparisons. Then the organoleptic tests using weighted adjectives may be used. These are of two types: the "hedonic" and the "quantitative."

Let us start with the "hedonic" scales (1). To be "hedonic" a scale does not necessarily have to have nine subdivisions as is sometimes thought. It is called "hedonic" because it records the pleasantness of sensations. It does this in terms of "like, dislike"; "good, poor"; "acceptable, unacceptable" and the like. It is a very useful device for testing consumer preferences. It is not designed for following quantitative changes in a food. For instance, a judge who disliked meat cooked rare gave a lower score for juiciness to a rare steak than to one cooked well-done because she didn't like a steak which was "too juicy." The hedonic scale gave her the opportunity to record her personal dislike but imagine the confusion which would attend any effort to compare such scores with any of the physical or chemical tests for juiciness! For those comparisons a more quantitative scale of very juicy, juicy, dry, very dry would be needed.

At one time Texas was one of several stations which had contracts on beef cookery. The rating scale was of the hedonic type because the adjectives used for scoring were: excellent, very good, good, medium, poor, very poor and unacceptable. In these tests for flavor, the judges rated braised steaks higher than broiled steaks! It is true that Home Economists have taught that the flavor of broiled or roasted meat is better than that of braised meat, but that doesn't mean that all members of the population agree with them. This incident points up to the need for consumer preference studies. It also indicates that serious difficulty might be encountered if hedonic scales are employed in trying to isolate the chemical causes of flavor differences. Let's do both kinds of studies but do them separately so that the objectives do not become confused.

These examples indicate some of the shortcomings of hedonic scales in studying possible changes in eating quality caused by chemical and physical changes in the meat as it cooks. More quantitative records are obtained when tenderness, for instance, is rated in such terms as "very tender, tender, tough, very tough."

At the Texas Station, we have found that tenderness exhibits scoring problems peculiar to it. It is likely that juiciness and flavor also have scoring problems peculiar to them. In subjective tests such as those mentioned above, tenderness has been thought of as having only one component. Yet, it has been recognized for more than 50 years that tenderness is influenced by two structures in meat, connective tissue and muscle fibers; that these two structures are not uniformly distributed among all muscles; and that some cooking methods may tender one component while toughening the other. Perhaps this may be one reason it has been difficult to associate subjective tenderness ratings with supposedly pertinent chemical or physical changes in the meat. Apparently still further improvements in scoring are needed to permit close association with other tests so that we may learn the causes of tenderness or toughness.

The inadequacy of one score for tenderness has been especially noticeable in my laboratory because of the variety of muscles and cooking conditions which we use routinely. We take one-muscle steaks from Longissimus dorsi in loin because this is a so-called "tender" muscle with supposedly little connective tissue, and from Biceps femoris in bottom round because it is a so-called "less tender" muscle with much connective tissue. Each of these two muscles is cooked under four conditions as follows: dry heat is applied by oven-broiling to two internal temperatures, 61° C. (rare) and 80° C. (well-done); while moist heat is applied by braising to two internal temperatures, 85° C. (medium-rare) and 100° C. plus holding there for 25 minutes (very well-done). Thus, at each judging session the panel must test eight samples of meat. These samples usu

ally differ widely in tenderness components. In the eight samples presented to our judges at one session, there may be many combinations of tenderness and toughness of muscle fibers and connective tissue. A confusing situation indeed for a judge with only one kind of tenderness score to assign to each sample! And, it is equally confusing to the research worker who tries to relate such scores to other tests!

If we are to progress deeper into the chemical causes of tenderness or toughness, we need precise descriptions of tenderness differences. It is especially important to separate toughness caused by connective tissue from that caused by muscle fibers. The results of the first such attempt in my laboratory were published in Food Research in 1957 (2). An improvement of the technique is described in another paper to be published in the September number of Food Research (3). Still other modifications are being considered.

The idea for scoring various components of tenderness came as I tasted cores after they had been sheared in the Warner-Bratzler shearing device. Samples from rare steaks are likely to be soft and juicy and may be swallowed before tenderness of either the muscle fibers or the connective tissue has been assessed. People who enjoy rare meat may almost be said to "drink" their meat. Thus, it is very important that each sample be chewed thoroughly. Records of juiciness and softness may be indicative of how quickly a judge would be inclined to swallow the sample had he not been told to masticate it thoroughly. During thorough mastication it is rather easy to distinguish connective tissue from muscle fibers. In bottom round steaks during thorough mastication the muscle fibers may disintegrate and leave a rather large mass of hard connective tissue. This is called "gristle" by home makers and must be either gulped or discarded. Tenderness of muscle fibers appears to be a very complex characteristic. The adjectives used to describe it might differ among different laboratories. Certain adjectives might be especially appropriate with one set of variables while other variables would need other adjectives. Under some of our conditions a mealy or powdery characteristic was most outstanding. We have called it friability at times and crumbliness at other times. We are still searching for a better term.

Table 1 shows the rating scales used by our judges for one series of tests. The characteristics which the judge looked for are given at the top: juiciness, and the various components of tenderness: softness, friability of muscle fibers, and tenderness of connective tissue. A rating scale of 11-points was used at that time with adjectives and a space between most of them for finer discrimination. The adjectives were arranged so that the highest score was given to the juiciest and softest meat, the most friable muscle fibers and the tenderest connective tissue. Notice that a high score does not mean highest acceptability in all cases. I, personally, do not enjoy eating meat which is "extremely friable" even though it may be cut with a fork. Those experimenting with enzymes as tenderizers might find that scoring for this component in addition to tenderness of connective tissue would give useful information.

Selecting and training judges is especially important with this method of scoring. Because we use only those data obtained when each judge is present, availability for judging rates high as a requirement. Knowledge of the structure of meat is essential. Training is slow and rather tedious. It is not easy to provide meat with the extremes of each characteristic. Most judges need considerable time to learn to detect the various components. We allow 20 or more training sessions. Early in the training period we sometimes sit around one table. I describe my sensations, then invite agreement, disagreement, questions and comments. When it seems that each potential judge is able to distinguish among the various characteristics, we have a few more training sessions in the individual booths—no talking. Meat is not homogenous and absolutely perfect agreement among all judges must not be expected. The sample for judging is small and all of it is placed in the mouth at one time. There is

TABLE 1

Adjectives Used in Scoring Meat and Associated Weightings
(1956 Data)

Weighting of Adjectives	Adjectives Used in Scoring			
	Juiciness	Softness	Friability of Muscle Fibers	Tenderness of Residual Connective Tissue
10	Extremely juicy	Extremely soft	Extremely friable	No. C.T. felt
9	Very juicy	Very soft	Very friable	Tiny amt. of soft C.T.
8				
7	Juicy	Soft	Friable	Small amt. of fairly soft C.T.
6				
5	Neither juicy nor dry	Neither hard nor soft	Moderately friable	Small amt. of firm C.T.
4				
3	Dry	Hard	Slightly friable	Medium amt. of firm C.T.
2				
1	Very dry	Very hard	Very slightly friable	Large amt. of hard C.T.
0	Too dry to swallow	Too hard to chew	Not friable	Residue very hard

no chance for second guessing.

Data obtained in my laboratory for each of the components have shown wide ranges within a muscle, as might be expected, because of the various conditions of cooking: broiling rare and well-done and braising medium and well-done. Table 2 shows the range of steak averages obtained within each muscle in one test. Note the wide ranges for loin in the first column and for bottom round in the second column.

The close association between scores for juiciness and tenderness has been noted by many workers. For this reason, the correlation between juiciness score and four of our variables may be of interest. Table 3 shows these co-efficients.

Juiciness score was more highly correlated with weight loss than with any of the other variables and the coefficients were similar for the two muscles. Since the weight loss was largely water, this close relationship was expected. The coefficients for juiciness score versus softness score were moderately high and were similar for the two muscles. Thus, the steaks which

TABLE 2

Ranges for 6 Variables in Loin and Bottom Round Steaks
(1956 Data)

Variables	No. of Steaks in Each Group*	Ranges	
		Loin	Bottom Round
Weight loss (%)	220	9.0 - 40.3	10.7 - 45.3
Juiciness score	220	1.5 - 10.0	2.0 - 9.8
Softness score	220	2.0 - 9.0	3.5 - 8.5
Friability score	220	1.3 - 9.8	4.0 - 9.8
Connective tissue score	220	5.3 - 10.0	1.5 - 10.0

*One steak by each of 4 methods of cooking x 55 animals = 220 steaks.

lost the least weight and were the softest tended to be the juiciest. These results are in line with traditional ideas.

The coefficients for juiciness score versus each of the other variables were considerably different for the two muscles. The drier steaks tended to be more friable than the juicier ones but this association appeared to be closer in bottom round than in loin steaks. Since the juiciest steaks were rare and the driest ones were very well-done this brings into question some of the older ideas about toughening of the meat proteins by heat, particularly the familiar statement "high temperatures toughen protein." The effect of higher internal temperatures on tenderness of muscle fibers may be much more complex than has been supposed. Only future work can tell us whether the change to "not friable" in some steaks or to "extremely friable" in others is associated with changes in the hydration envelope of the protein (its extent and rigidity) or whether the characteristic of extreme friability is associated also with alteration of protein structure.

The juicier steaks tended to have the tougher connective tissue. This relationship was low and not significant in loin steaks, where the connective tis-

TABLE 3

Correlation Coefficients for Each Pair of Variables Within
Loin Steaks and Within Bottom Round Steaks
(1956 Data)

Variables	No. of Steaks in Each Group*	Correlation Coefficients	
		Loin (L. dorsi)	Bottom Round (B. femoris)
Juiciness score versus:			
Weight loss	220	-.848***	-.896***
Softness score	220	.612***	.593***
Friability score	220	-.210***	-.673***
Connective tissue score	220	-.118 n.s.	-.764***

*One steak by each of 4 methods of cooking x 55 animals = 220 steaks.

n.s. and *** indicate significance above 5% level and at 0.1% levels, respectively.

sue in these animals was tender by all methods of cooking, but it was rather high and significant for bottom round steaks, where the connective tissue was tough at 61° C. and tender at 100° C.+. Thus, the traditional concept of tendering of heavy connective tissue in round steaks on longer cooking appears to be upheld by these tests.

Thus, when we attempt to untangle juiciness score from tenderness we find that softness is the only one of the three tenderness components which is positively correlated with juiciness. Moreover, Longissimus and Biceps behave similarly for juiciness versus softness, but the two muscles behave differently for juiciness versus friability of muscle fibers and for juiciness versus tenderness of connective tissue. This suggests that there are fundamental differences between the two muscles, not only in connective tissue but in muscle fibers as well. The possibility that chemical differences are responsible should lead to further work.

Basic work on connective tissue and muscle fibers is being done in many laboratories using chemical, histological, x-ray and electron microscope techniques. The literature is voluminous and stimulating. There are almost unlimited opportunities for relating research in these fields to subjective tests for tenderness; particularly, if tenderness can be separated into components which are likely to be pertinent.

In previous attempts to relate collagen content to one score for tenderness we were baffled because the scores for tenderness were so similar for the two muscles, while the collagen content differed widely (4). Only when the new meth od of scoring connective tissue was used, could the relationship of this chemical component to tenderness be shown. Miss Lanell Irvin, of our group, has determined the collagen content of these two muscles by a modification of the Lowery (5) procedure. Figure 1 shows the collagen content (in left margin) plotted against the scores for tenderness of connective tissue in steaks broiled rare. The solid dots, mostly at the upper left, represent biceps and the open circles, mostly at the lower right, represent Longissimus. In general, Biceps had tougher connective tissue (average score 3.0 vs 9.3) and greater collagen content (2.45 vs 1.42) than did Longissimus (6).

Basic research is showing that there are several types of collagen in connective tissue organs such as skin and tendon; and that they may be determined chemically as well as by histological and other means. Perhaps these types of collagen are present also in the connective tissue within a muscle. If they are present, do their proportions vary with breeding, age, management, processing procedures, or methods of cooking and are these possible variations in type of collagen related to tenderness of connective tissue? We are most interested in these questions at the Texas Station and hope to add a well-trained protein chemist to our co-operative group to help us answer these and other questions concerning the relationship of meat proteins to tenderness.

Dr. Cartwright (7) told you last year about the way we are relating our tenderness work to breeds and sires and so I shall not discuss that aspect of our work. But I have brought some slides on carcass grades and fatness as further examples of co-operation (8,9). In the interest of brevity, data on braising to 85° C. (medium-rare) are omitted because of their similarity to those for broiling to 80° C. (well-done). Data on the different cuts are shown in Figures 2-5.

Figure 2 shows carcass grade plotted against scores for tenderness of connective tissue of loin steaks. Note that the connective tissue was tender and apparently was not affected by method of cooking (broiled rare at top, broiled well-done in center and braised very well-done at bottom). Nor was it affected to any great extent by carcass grade within any of the methods of cooking. Figure 3 shows corresponding data for bottom round. Note that the connective tissue increased in tenderness with more thorough cooking from broiled rare at top, to broiled well-done in center, and to braised very well-done at bottom.

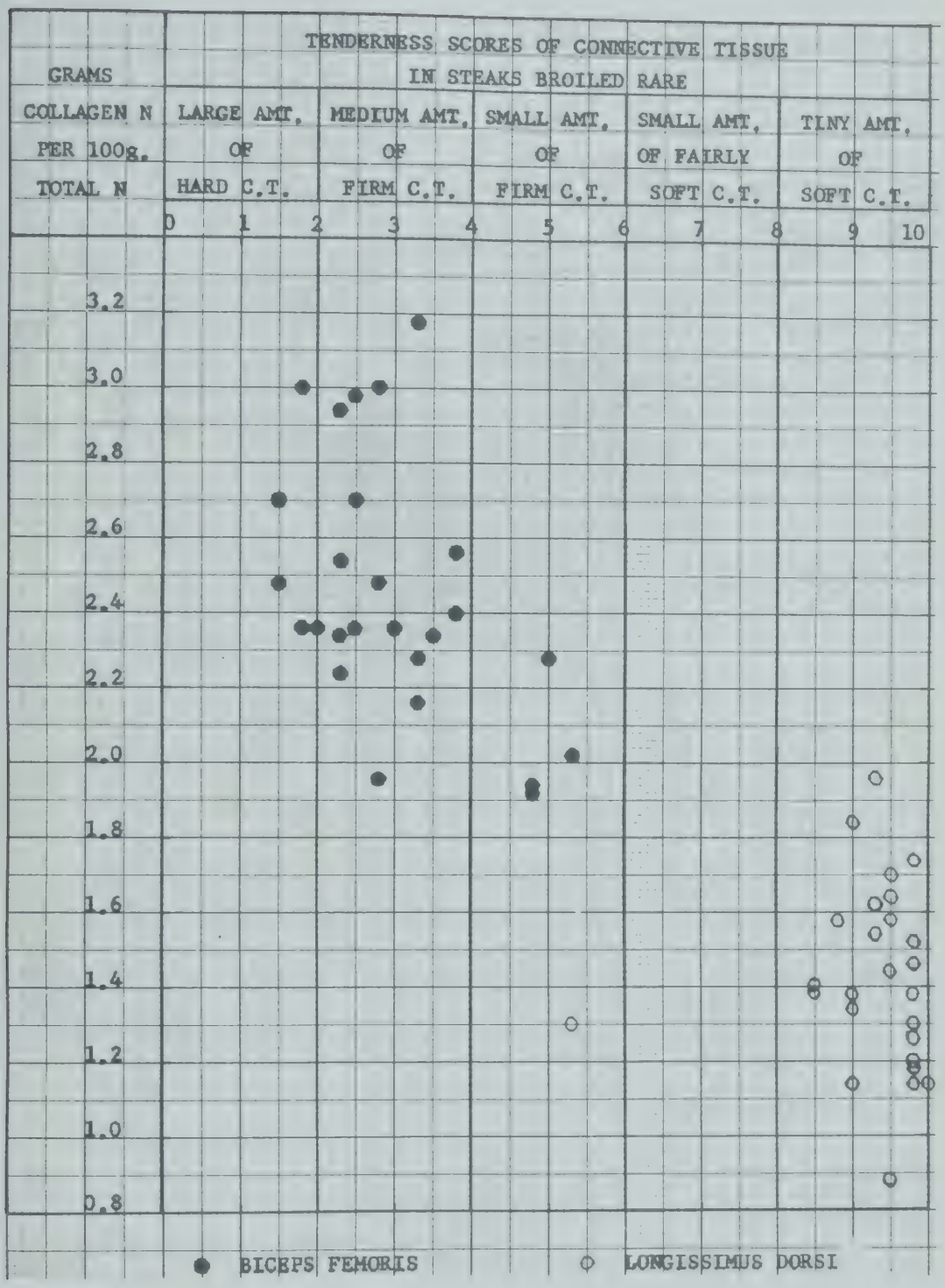
TENDERNESS SCORES OF CONNECTIVE TISSUE IN STEAKS BROILED RARE
GRAMS COLLAGEN N PER 100g. TOTAL N
LARGE AMT. OF HARD C.T.
MEDIUM AMT. OF FIRM C.T.
SMALL AMT. OF FIRM C.T.
SMALL AMT. OF FAIRLY SOFT C.T.
TINY AMT. OF SOFT C.T.
0 1 2 3 4 5 6 7 8 9 10
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
BICEPS FEMORIS
LONGISSIMUS DORSI

Figure 1.

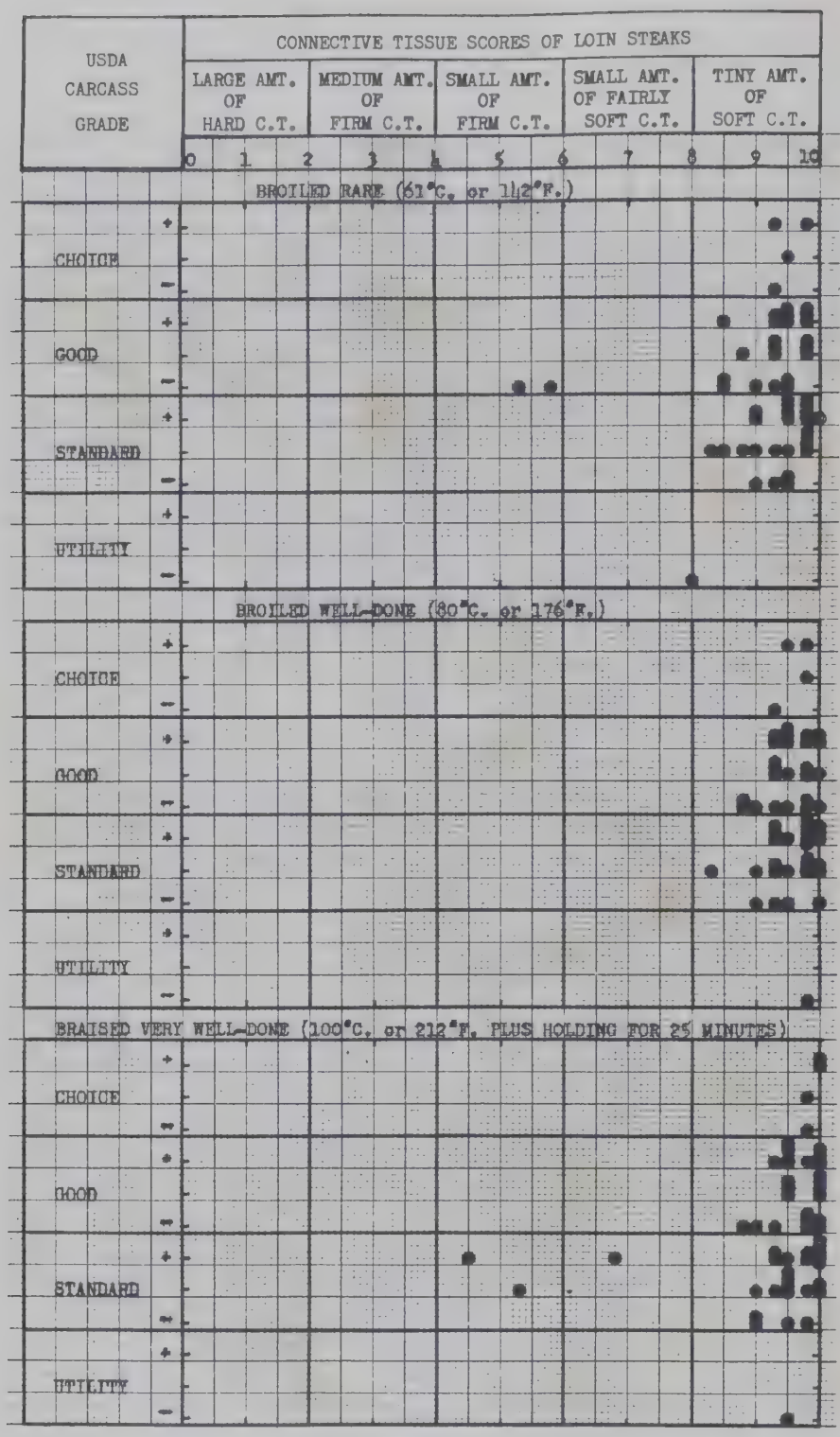
USDA CARCASS GRADE
CONNECTIVE TISSUE SCORES OF LOIN STEAKS
LARGE AMT. OF HARD C.T.
MEDIUM AMT. OF FIRM C.T.
SMALL AMT. OF FIRM C.T.
SMALL AMT. OF FAIRLY SOFT C.T.
TINY AMT. OF SOFT C.T.
0 1 2 3 4 5 6 7 8 9 10
BROILED RARE (61°C. or 142°F.)
CHOICE + −
GOOD + −
STANDARD + −
UTILITY + −
BROILED WELL-DONE (80°C. or 176°F.)
CHOICE + −
GOOD + −
STANDARD + −
UTILITY + −
BRAISED VERY WELL-DONE (100°C. or 212°F. PLUS HOLDING FOR 25 MINUTES)
CHOICE + −
GOOD + −
STANDARD + −
UTILITY + −

Figure 2.

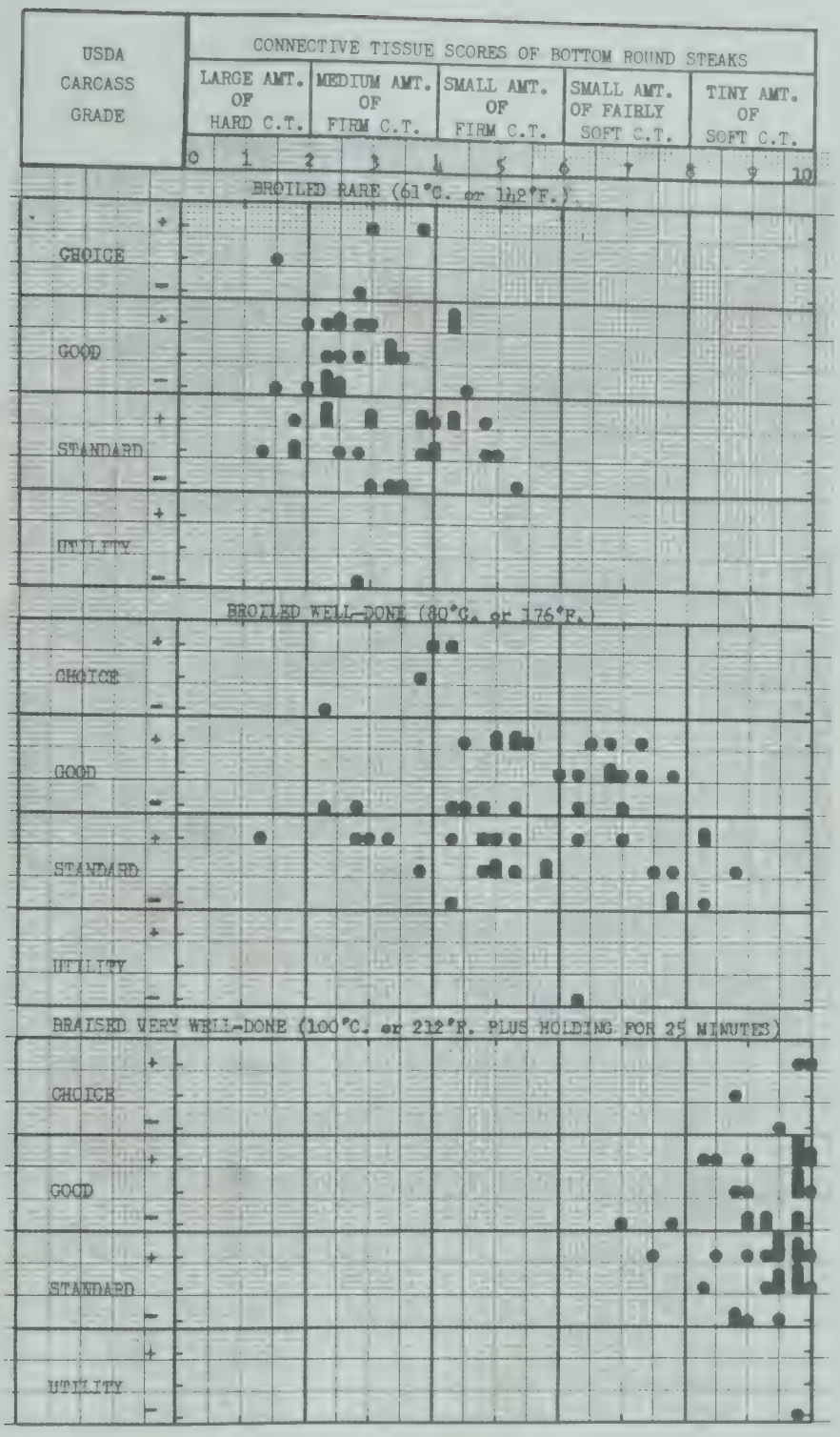
USDA CARCASS GRADE
CONNECTIVE TISSUE SCORES OF BOTTOM ROUND STEAKS
LARGE AMT. OF HARD C.T.
MEDIUM AMT. OF FIRM C.T.
SMALL AMT. OF FIRM C.T.
SMALL AMT. OF FAIRLY SOFT C.T.
TINY AMT. OF SOFT C.T.
0 1 2 3 4 5 6 7 8 9 10
BROILED RARE (61°C. or 142°F.)
BROILED WELL-DONE (80°C. or 176°F.)
BRAISED VERY WELL-DONE (100°C. or 212°F. PLUS HOLDING FOR 25 MINUTES)
CHOICE
GOOD
STANDARD
UTILITY
+
-

Figure 3.

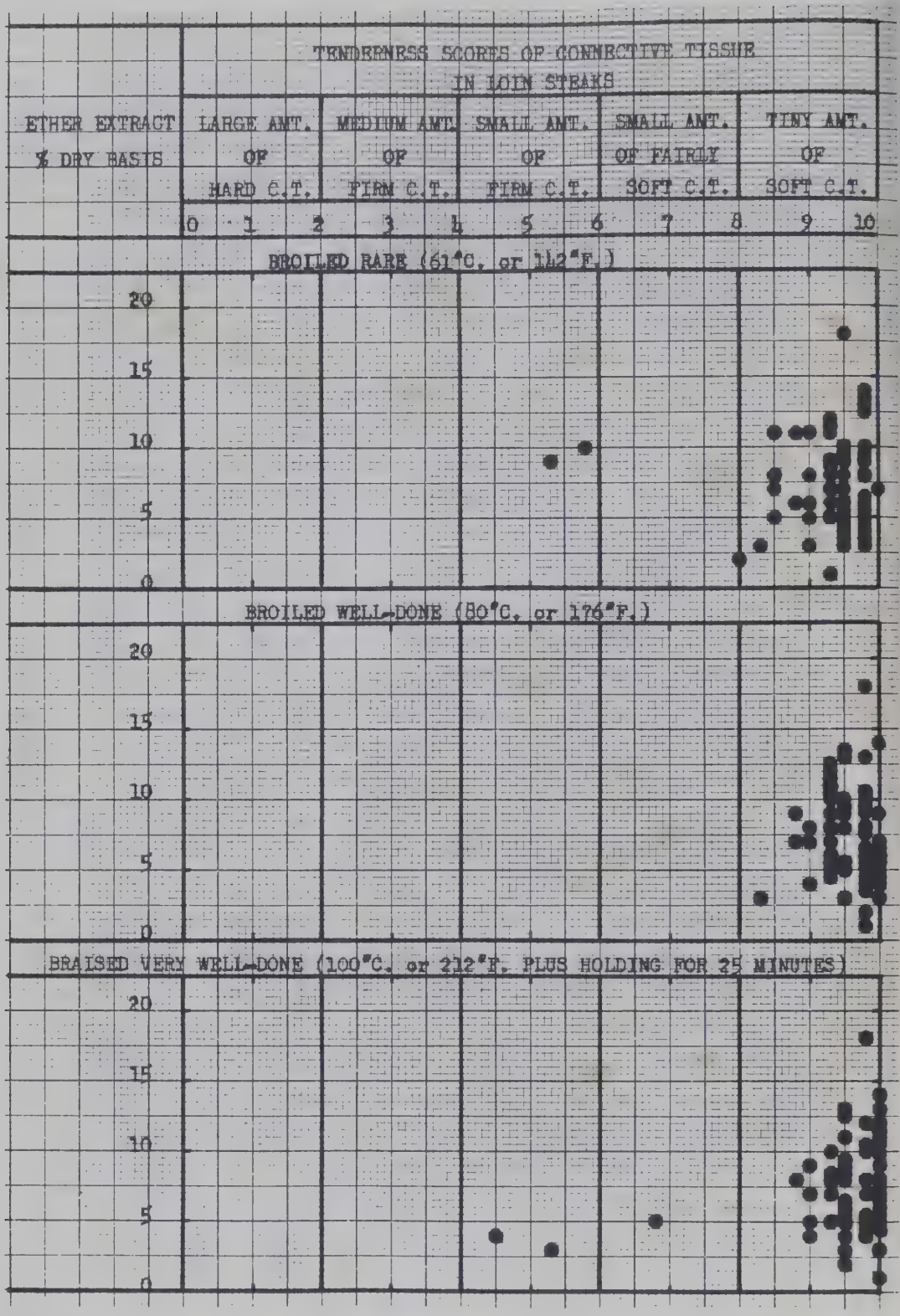
TENDERNESS SCORES OF CONNECTIVE TISSUE IN LOIN STEAKS
ETHER EXTRACT % DRY BASIS
LARGE AMT. OF HARD C.T.
MEDIUM AMT. OF FIRM C.T.
SMALL AMT. OF FIRM C.T.
SMALL AMT. OF FAIRLY SOFT C.T.
TINY AMT. OF SOFT C.T.
0 1 2 3 4 5 6 7 8 9 10
BROILED RARE (61°C. or 142°F.)
BROILED WELL-DONE (80°C. or 176°F.)
BRAISED VERY WELL-DONE (100°C. or 212°F. PLUS HOLDING FOR 25 MINUTES)
20
15
10
5
0

Figure 4.

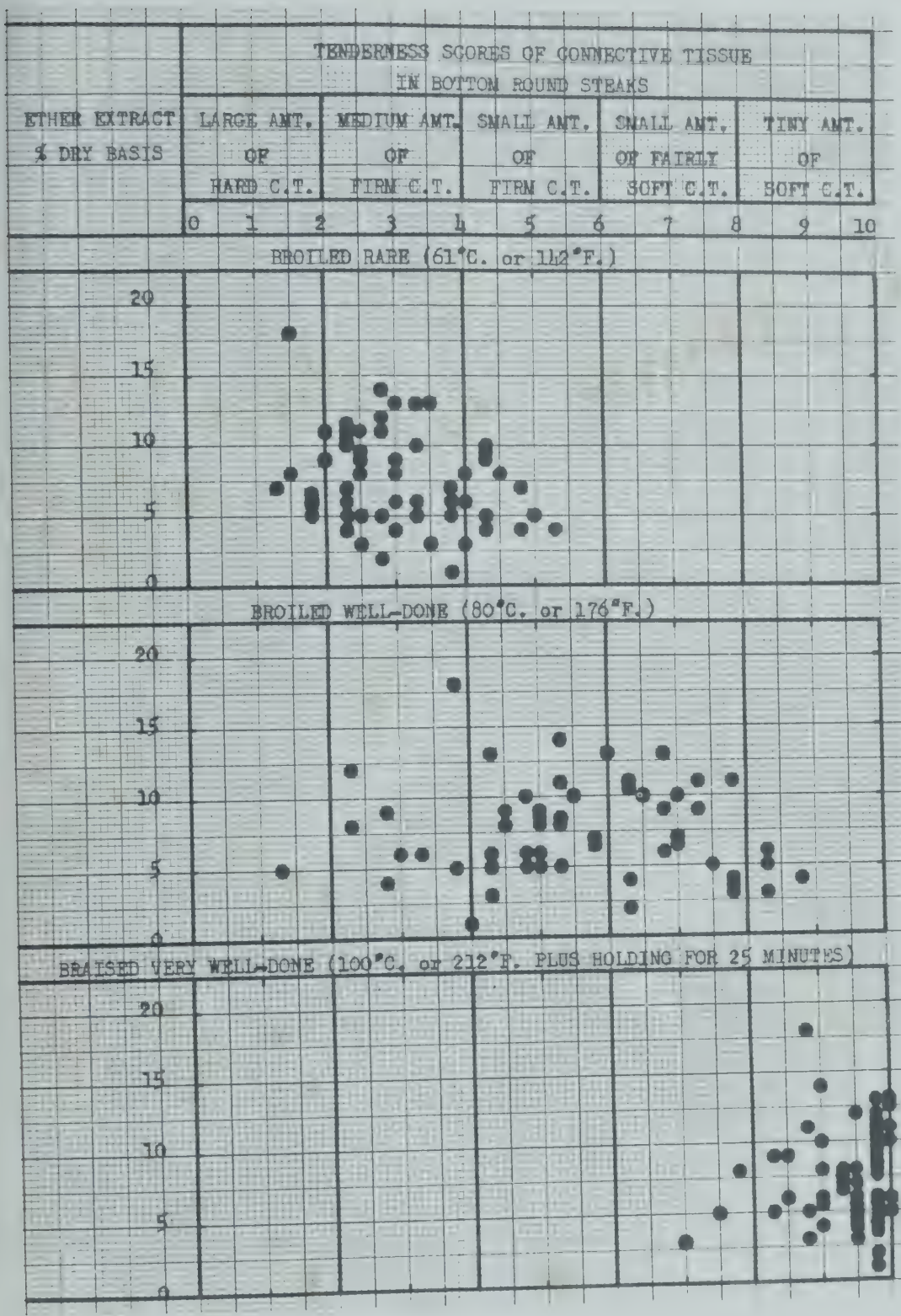
TENDERNESS SCORES OF CONNECTIVE TISSUE IN BOTTOM ROUND STEAKS
ETHER EXTRACT % DRY BASIS
LARGE AMT. OF HARD C.T.
MEDIUM AMT. OF FIRM C.T.
SMALL AMT. OF FIRM C.T.
SMALL AMT. OF FAIRLY SOFT C.T.
TINY AMT. OF SOFT C.T.
0 1 2 3 4 5 6 7 8 9 10
BROILED RARE (61°C. or 142°F.)
BROILED WELL-DONE (80°C. or 176°F.)
BRAISED VERY WELL-DONE (100°C. or 212°F. PLUS HOLDING FOR 25 MINUTES)
20
15
10
5
0

Figure 5.

The higher carcass grade did not appear to increase tenderness of connective tissue within any method of cooking. Thus in these data from 55 animals, tenderness of connective tissue appeared not to be a problem in loin steaks from any of these carcass grades. Moreover, in bottom round steaks, tenderness of connective tissue appeared to be affected more by method of cooking than by carcass grade.

Our fatness data will be illustrated by ether extract, a chemical estimate of marbling. Figure 4 shows the percentage of ether extract plotted against scores for tenderness of connective tissue in loin steaks. Note that in none of the methods of cooking was the higher ether extract associated to any great extent with higher scores for tenderness of connective tissue. Figure 5 shows the corresponding data for bottom round. Note that in none of the methods of cooking is there a close relationship between heavier marbling and higher scores for tenderness of connective tissue. Thus, the relationship between tenderness of connective tissue and carcass grade or fatness does not appear to be as close as has been supposed in the past. Data from two other years show the same trends. In two of the three years there has been a complete change in personnel on the panel, and in personnel doing the cooking. Animals also varied each year. Similar trends under these conditions give us greater confidence in the reliability of these trends.

Perhaps some of you will want to adapt the technique of dividing tenderness into two or more components to your own situation. I would like to suggest that you use as simple a rating scale as possible in your first test. This allows the worker in charge to experience characteristics exhibited by muscle fibers under his conditions. Then he can determine which one is or which ones are the most distinctive and useful for his purpose. As we learn more of the physico-chemical make-up of the muscle fiber we may wish to search for other pertinent characteristics.

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XIV

RECENT DEVELOPMENTS IN THE EXPERIMENTAL ANALYSIS OF BEHAVIOR

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In his "An Introduction to the Study of Experimental Medicine," Claude Bernard wrote: "I am convinced, in experimental sciences that are evolving, and especially in those as complex as biology, discovery of a new tool for observation or experiment is much more useful than any number of systematic or philosophic dissertations. Indeed, a new method or a new means of investigation increases our power and makes discoveries and researches possible which would not have been possible without its help" (4, p. 171).

Bernard's point certainly is well demonstrated by B. F. Skinner's discovery of frequency of responding as a dependent variable in the investigation of the factors controlling voluntary behavior (43,45). In 1930, Skinner (35) described an experimental method that, with various modifications by many subsequent workers, has become "a fixture in modern experimental research on behavior" (23, p. 42). The paper described a way of measuring the rate of eating by a white rat. The method involved: 1) an experimental cage containing a device for giving a small pellet of food to a hungry rat each time the animal pushed open the swinging door of a foodbin at one end of his chamber; and 2) a mechanism for automatically recording the rate of panel pushing or eating. Today, as in Skinner's original studies, the rate of responding usually is recorded as a cumulative curve on a modified kymograph. A pen moves across a paper tape driven by a slowly revolving cylinder. The pen, driven by a ratchet device, moves in the vertical direction a short uniform distance with each response. The resulting sloping line therefore is proportional to the rate of responding.

Figure 1 is an illustration of the construction of a cumulative record of behavior. The stepwise nature of the record is obvious in this figure because of the excessively large time and response units selected for illustrative purposes. In contemporary work involving many different species, appropriate paper speeds and unit steps are chosen in such a way that the rates to be studied give convenient slopes. It may be noted that at no point does the experimenter intervene for purposes of interpretation of the records generated by a cumulative recorder. The cumulative recording data presented in this paper are photographic reproductions of records made by the animals themselves (15,36).

In laboratory experiments selection of a response is based on the following considerations: 1) The response should be objectively measurable. 2) The behavior should be able to be executed easily by the subject. 3) The experimental subject should be able to respond repeatedly without fatigue (45).

In experiments with pigeons, the birds are trained to strike at (peck) a small translucent plastic key mounted at the height of the beak in one of the walls of a small cage as shown in Figure 2. In some experiments a bird may make as many as 70,000 pecks during a 4-1/2 hour experimental period (15). With pigeons, rates of key-pecking may vary between zero and 15 pecks per second.

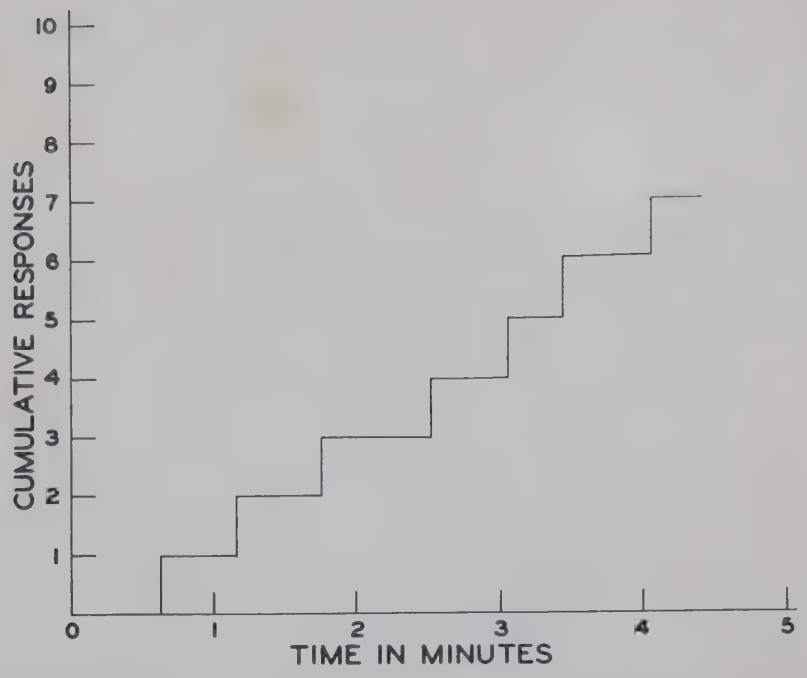

Figure 1. Illustration of the construction of a cumulative record.

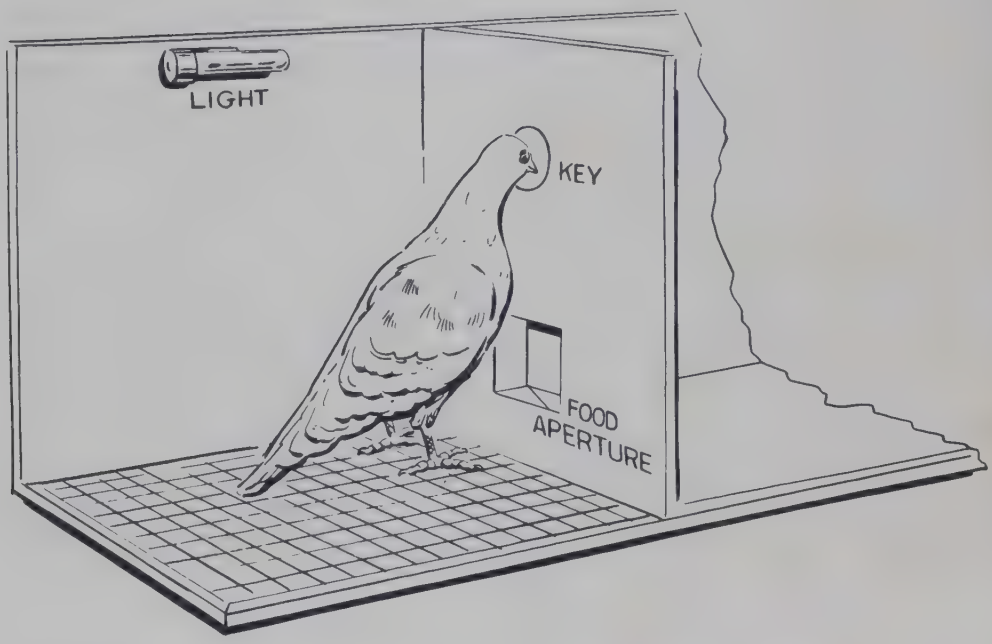

Figure 2. Illustration of an experimental cage used for operant-conditioning research with pigeons.

Figure 3 is an illustration of a cumulative record as "drawn by" an animal. The curve shows the cumulative record of a rat on a variable-interval schedule of reinforcement (reward) (15). In this particular experiment the animal pressed a small lever mounted in one of the walls of a small cage as illustrated by Figures 4 and 5. Pressing of the lever was rewarded by a small food pellet at various different intervals since the previous reward. During the periods between rewards the animal was free to press the lever, however, without being reinforced. In this illustrative experiment the intervals between

Figure 3. Performance of a rat under variable-interval reinforcement.

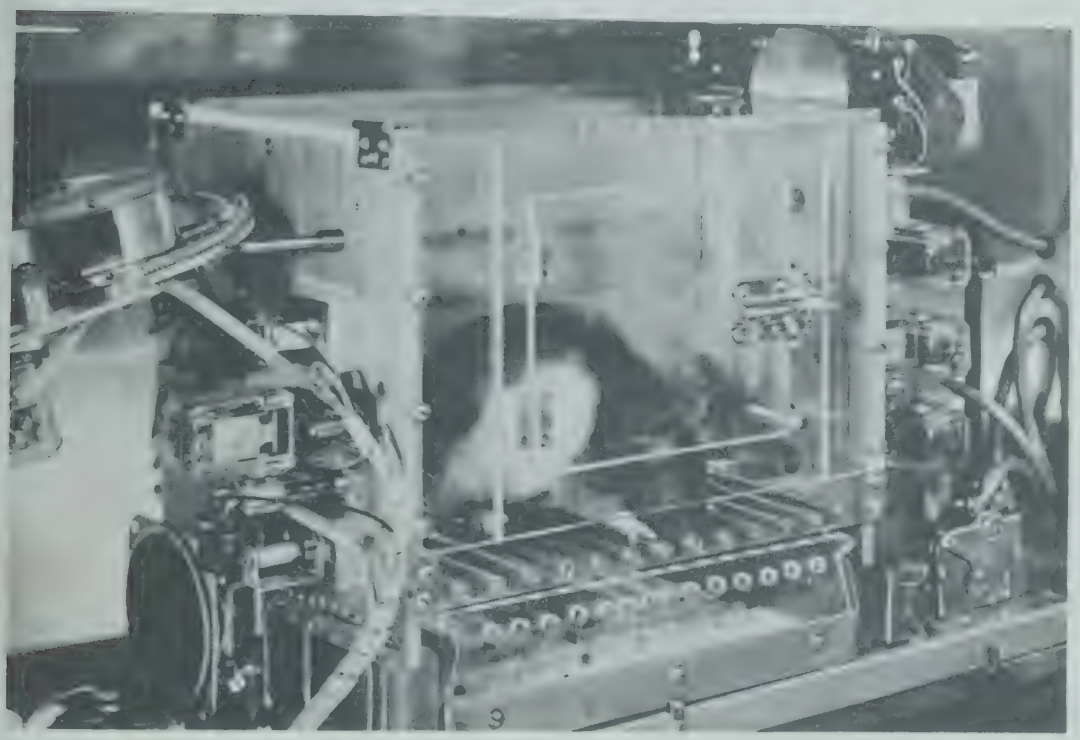

Figure 4. An experimental cage used for research with rats.

payoffs ranged from 5 to 120 sec. The mean interval between rewards was 1 minute. The scale of the record is indicated by the insert in the figure, which shows a small set of co-ordinates containing some representative slopes.

This experiment, as all contemporary work, was programmed automatically by means of electrical relay circuits. Data were recorded automatically by means of running-time meters, impulse counters and a cumulative recorder. Figure 6 shows a photograph of experimental control and recording equipment used at the Lilly Research Laboratories.

The consequences of Skinner's initial modest and seemingly trivial experiment have been staggering. Ever since 1930, and at a positively accelerated rate the end of which is not yet in sight, hundreds of rats and pigeons, as well

as mice, turtles, chimpanzees, fish, cats, dogs, college students, mentally defective persons, psychotic patients and naval trainees, have been pushing doors, pressing levers, nosing plastic disks, and pulling all sorts of switches, unwittingly producing cumulative records.

Figure 5. A rat in the act of pressing a lever.

In all these experiments the behavior of the subjects is controlled by the consequences. In the experiment with the rat that was previously used as an illustration, the consequence of pressing the lever was the occasional pellet of food received by doing so. Such consequences of behavior, ranging from water, candy and chicken feed to "a pat on the back by the boss," are some of the most powerful factors that alter the rate or frequency of voluntary behavior. With this type of behavior the organism usually acts upon his environment, thus producing changes in it. Skinner proposed the term "operant behavior" because the organism operates on his environment (36,38). Every step towards a door reduces the distance between the walker and the door, to use a down-to-earth illustration. These changes in the environment, produced by the organism itself, subsequently change the organism, affecting its future behavior. Because of these feed-back effects of what in daily life are usually called rewards and punishments, the present area of research might be thought of as a kind of experimental cybernetics.

The data in Figure 3 illustrate the fact that responding can be maintained by reinforcing the behavior only intermittently. Many fascinating phenomena have been discovered by the use of "intermittent reinforcement" procedures. A large volume of research dealing with the effects of various "schedules of reinforcement" has recently been published (15).

Figure 7 shows a typical performance of a rat on a frequently used schedule of reinforcement. In this experiment the animal was reinforced by the presentation of a measured amount (1/2 cc) of sweetened condensed milk by means of a dipper. The schedule of reinforcement was a 12-minute fixed-interval (FI) schedule (15); lever pressing was reinforced at intervals of 12 minutes. When a reinforcement was received, pressing the lever became in-

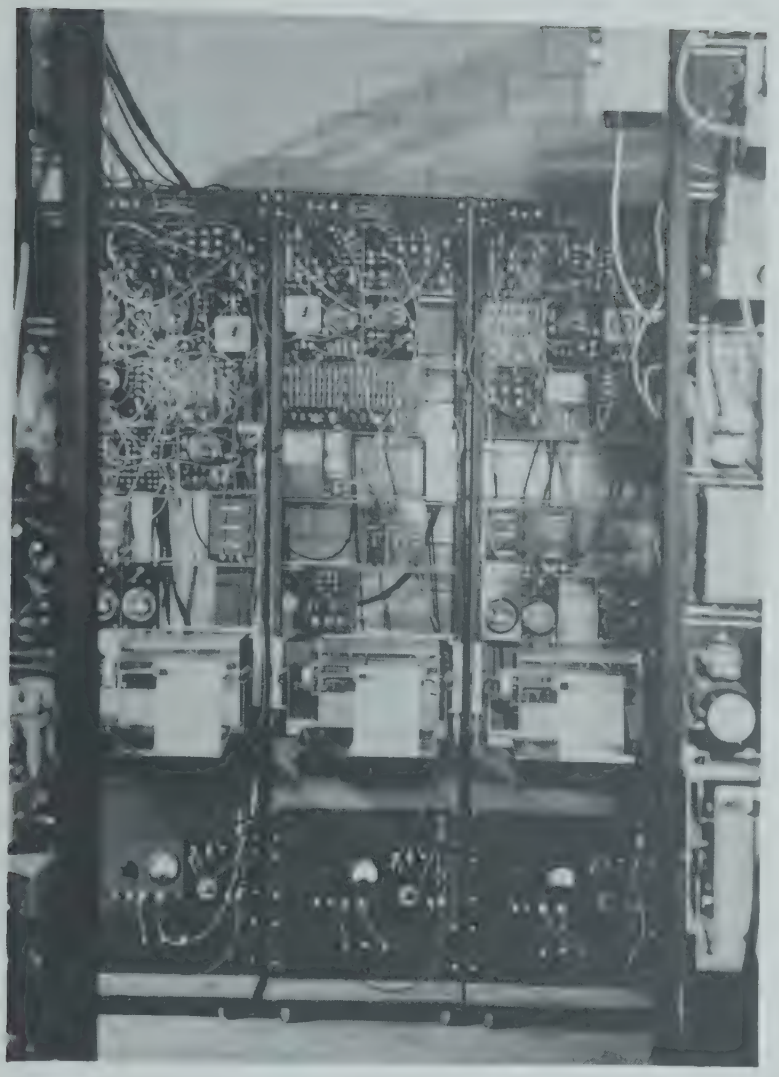

Figure 6. Controlling equipment used in research on animal behavior at the Lilly Research Laboratories.

effective until a 12-minute recycling timer ran out, closing the electrical circuit between the lever and the dipper. The dipper was accessible for a 10-second period, long enough to give the animal a chance to clean out the cup of milk. The reinforcement period was followed by a 5-minute time-out period during which all the lights in the cage were turned off. At the end of this 5-minute break, a light came on above the lever and the 12-minute timer was started again. Figure 7 shows a sample portion of the cumulative record obtained during a session after a considerable amount of training. The animal had been previously exposed for more than 100 hours to the schedule.

Figure 7. Characteristic performance by a rat under fixed-interval reinforcement.

In this experiment the cumulative recorder pen was automatically reset to the bottom of the recorder at the end of each 5-minute time-out period after each reinforcement. Reinforcements are indicated by small oblique marks on the record, like the one near the arrow. In the short sample of the performance shown in Figure 7 the onset of the light above the lever started at "a." The first reinforcement was not given until 12 minutes later (at the arrow). It can be seen that the animal started responding at a very low rate that, as time passed, accelerated into a fairly stable terminal rate at "b" maintained until

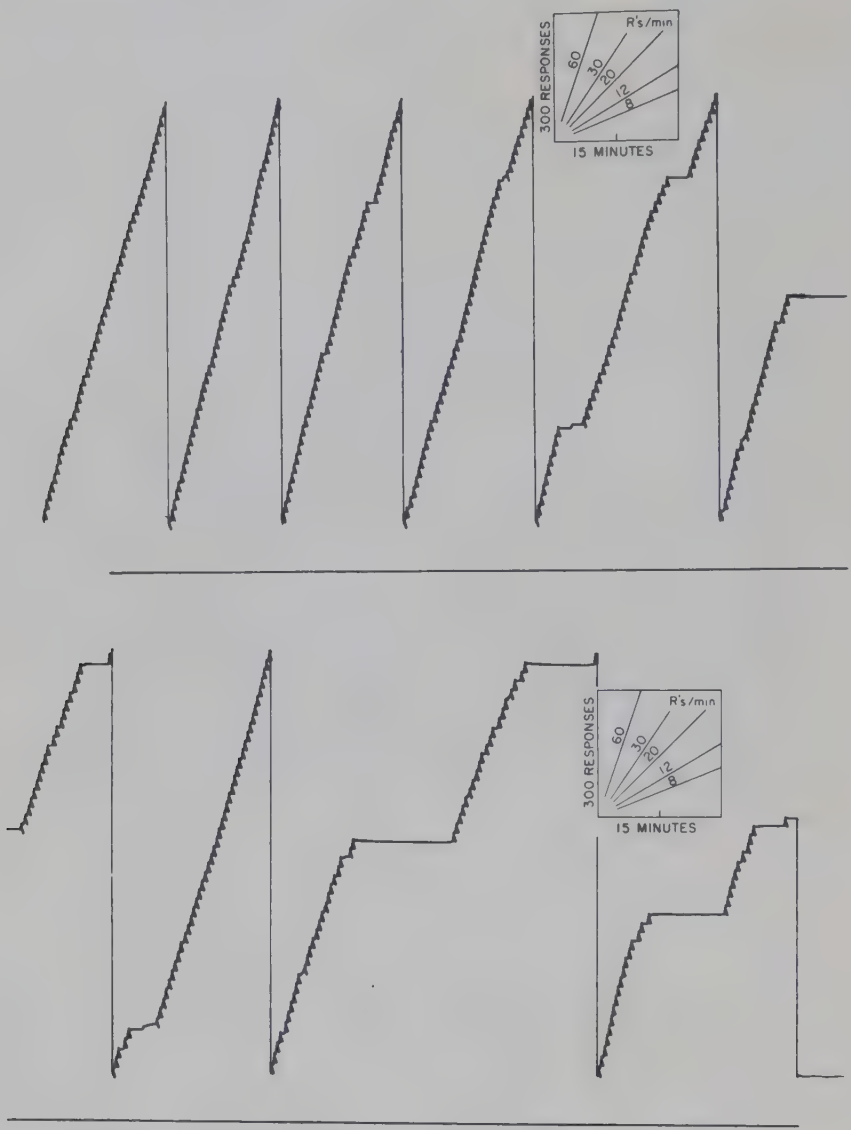

Figure 8. Typical performance by a monkey under fixed-ratio reinforcement. Reinforcements occurred at the vertical marks on the record.

reinforcement occurred. During the 5-minute time-out periods the animal did not respond at all. This is indicated by the flat horizontal stretch of record that appears after each reinforcement. During the subsequent 12-minute fixed interval periods, responding did not start until a few minutes after onset of th light above the lever (at "d"), which coincided with the return of the pen to the baseline (at "e"). Abortive starts are made occasionally (at "f"). A smooth gradual acceleration with an occasional pause may be seen at "g." The overal characteristic pattern of performance shows fairly gradual, positively accelerated scallops in each fixed interval period (15,36). A characteristic perfor-

nance when the intervals between reinforcement are not fixed is shown in igures 3 and 9.

As P. Dews (13) has recently pointed out, the performance of an organism on a fixed-interval schedule, whether rat (36), pigeon (15), or human being (22), is not what one would expect. In discussing the effects of certain harmacological agents on the pecking rate of pigeons before a scientifically oriented lay audience, he explicitly emphasized that cumulative curves should be taken at their face value." One should "not sit in judgment of the ntelligence" of the animal in these experiments. The trained experimenter as learned to view his cumulative records with the same detached objectivity with which a physical chemist looks at an infrared absorption spectrum. One would not try to put oneself in the place of a molecule being irradiated o decide which wave lengths one would expect to be absorbed" (13).

It is also very easy to talk about the timing behavior of an animal under hese conditions of reinforcement. It might be pointed out, however, that it nakes no more sense to state that an animal is timing (or has a sense of ime) than it would be to say the same thing about a condenser in a resistor-condenser circuit. The only important questions concern the relevant experimental variables that control the performance.

The effects of piecework schedules, among others, have also been extensively investigated (15). Figure 8 shows the cumulative response record of a monkey pressing a lever for water as a reinforcement. The monkey is on piecework in the sense that only each 28th response is reinforced. Such a reward schedule is called a fixed-ratio (FR) schedule of reinforcement.

If one compares the curves of Figures 3 and 8 some interesting differences are apparent. Whereas the rat of Figure 3 works steadily at a fairly constant rate, the monkey works in spurts. Small but definite pauses are observable after each reinforcement for the animal on the ratio schedule. These pauses become more frequent and their duration increases as the number of rewards received increases. Satiation records of animals working on a variable-interval schedule of reinforcement as shown in the case of Figure 3 look quite different. Figure 9 shows the cumulative record of a rat on a one-minute variable-interval schedule pressing a lever for water as a reward. In this case the curve is negatively accelerated and levels off smoothly.

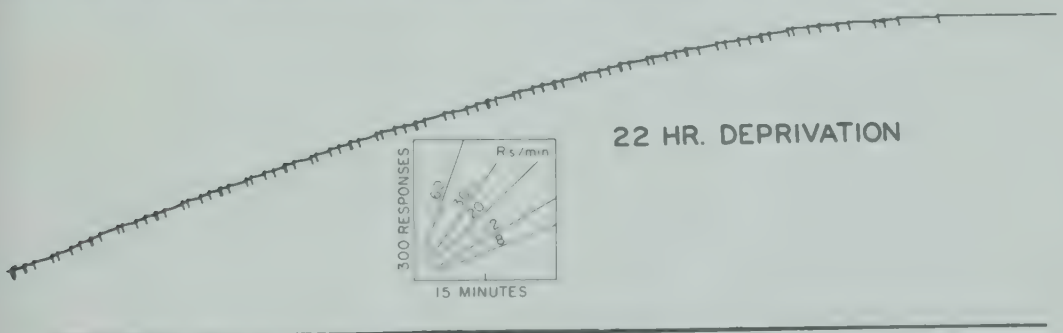

Figure 9. Performance of a rat under variable-interval reinforcement and low deprivation. Reinforcements occurred at the vertical marks on the record.

The difference in performance in the various experiments described so far are due to the particular schedules of reinforcement employed. They are not specific for a certain species or type of reward used. Similar results have been obtained with many different species and other reinforcements (13,15,36, 39,43,45).

The performance on a fix-interval schedule has shown itself to be a sensitive baseline for the investigation of the effects of drugs and other experi-

mental manipulations. Auerbach, Waisman and Wyckoff, for example, were able to demonstrate significant decreases in the frequency of appearance of the positively accelerated scallops in records made by rats on a 2-minute fixed-interval schedule after phenyl ketonuria had been induced in the anima by means of a special diet containing dl-phenylalanine and l-tyrosine (2).

Other experiments relevant to the field of nutrition have been described recently. Weiss (52) and Carlton and co-workers (10) independently worked out an operant-conditioning technique for studying the rewarding effects of warmth in a cold environment. Weiss used the 10-sec. activation of an infrared heat lamp as the reinforcement for pressing a lever by a rat. The ambient temperature of the box in some of these experiments was held at 0° C. Weiss has employed this particular technique for studying the effects of pantothenic acid deprivation of this so-called "thermal behavior" of the rat (53). His experiments have shown that rats deprived of food and of pantothenic acid are more sensitive to cold (as indicated by a higher rate of lever pressing) than animals deprived of food only. These experiments give only an indication of the potentialities of operant-conditioning research in this area. Only a few other experiments relevant to the field of nutrition have been performed (12). Anliker and Mayer (1), for example, have demonstrated that the eating behavior of obese mice is influenced in distinctive ways, depending on the kind of obesity involved. Some mice of a particular litter simply grow fat and by selective breeding obese strains can be produced. Normal mice can b made obese by injecting gold thioglucose or by damaging the hypothalamus. When these different types of obese animals are trained to obtain their food by depressing a lever on a small fixed-ratio (piecework) schedule (e.g., ever 25th press is reinforced), the kind of obesity can be identified by the patterns of responding produced on the cumulative recorders. The normal non-obese mouse eats a large part of his daily ration in a single period. For the rest of the day it may only press the lever occasionally, responding at a low overall rate. Mice poisoned with gold thioglucose and mice with hypothalamic lesions do not show the normal 24 eating-not-eating cycles but continue to respond a fairly steady rates, an indication that the mechanism which switches off eating is disturbed or not operating. Hereditary obesity is characterized by brie periods of a high rate of eating, separated by pauses of one to two hours (1,4

The phenomena of operant conditioning are not restricted to animals. As a matter of fact most of our daily activities involve operant behavior. Anyone in the possession of a watch with a large sweep second hand can demonstrate to himself some of the basic principles.

A lecturer standing in front of a fairly small audience makes an especia ly good subject. Many speakers let their eyes wander from person to person. Before "conditioning," the experimenter should determine the number of tim the speaker looks at him during a given period, e.g., 10 minutes. This allows one to calculate the pre-experimental control rate of the response involved. Subsequently one can start reinforcing this behavior. From now on, every time the speaker looks at him the experimenter should smile or nod, meanwhile keeping a record of the frequency of the speaker's behavior during suc cessive 5-minute intervals. It is not uncommon to triple or even quadruple the frequency of the "looking-at-the-experimenter" behavior within a 20-min ute period.

Laboratory studies of operant conditioning have not been limited to beha ior controlled by rewarding or pleasant consequences. One of the more frequent methods of controlling behavior, both in animals and in man, employs so-called aversive consequences, or punitive measures (38). In daily life suc "negative reinforcers" may range from physical abuse to fines and ridicule. A negative reinforcer can be defined as a behavioral consequence that induce organisms to act in order to escape from it or to prevent it from happening entirely (avoidance).

The use of electric shock in animal behavior research has been common practice for decades. However, laboratory techniques for generating stable rates of responding controlled by aversive consequences were not available until 1953. In that year M. Sidman disclosed a technique that opened up the entire field of avoidance behavior (32). An extensive experimental analysis of escape and avoidance behavior as well as of the effects of punishment is currently being pursued in several laboratories.

In Sidman's initial series of avoidance experiments, rats were given an electric shock through a grid floor at regular intervals unless a lever was depressed by the animal. Each lever depression reset the timer that controlled the shock and thus delayed its occurrence. If, for example, each response reset a 30-second timer, a minimum interval of 30 seconds was insured between avoidance behavior and shock. By continuously pressing the lever within 30-second intervals the rat would not get shocked at all. The time interval with which each response postpones the shock (the response-shock, or RS, interval) does not have to be equal to the time interval between shocks (the shock-shock, or SS, interval) when no responding occurs at all. As in Skinner's original experiment, Sidman's technique permits using the rate of responding as a continuous and direct indicator of the effects of experimental manipulations.

A sample of the lever-pressing behavior of a rat during a 21-hour long session is shown in Figure 10. Neither food nor water was available to the animal during the experimental session. The interval between shocks and the

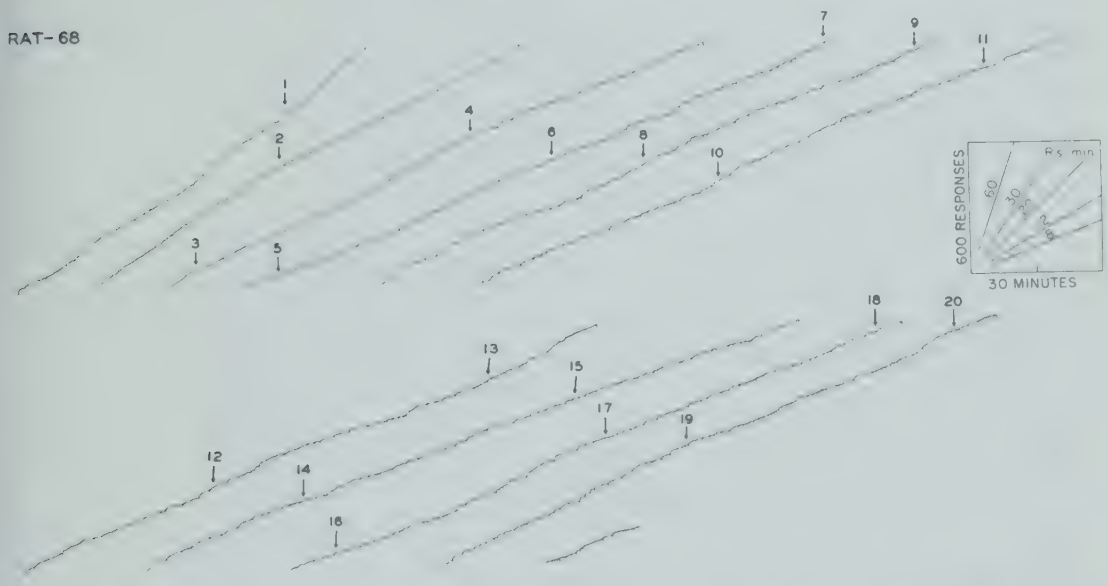

Figure 10. Performance of a rat that avoids shock by pressing a lever under the Sidman procedure. The numbers indicate successive hours. The occurrence of a shock is indicated by a vertical mark on the record.

time interval with which each press postponed the shock were 30 seconds. Since the slopes of the curves are the only relevant feature of a cumulative record, white space has been cut out of the original record and the separate segments have been brought closer together to facilitate reproduction. Shocks are indicated by the oblique marks on the record. An interesting and characteristic aspect of the performance is the warm-up effect at the beginning of the session. When the animal was first put into the experimental cage the rate of responding was low and the animal received a large number of shocks. This phenomenon occurs regularly at the beginning of a new session. The reasons for it are not yet very well understood. The cumulative record shows that the

number of shocks per hour increases as the session continues. The first si to eight hours, however, show a stable response as well as shock rate.

Much higher rates than the one shown by the rat of Figure 10 can be ge erated by certain modifications of Sidman's original procedure. Instead of r quiring the animal to make only one press to reset the shock timer, one can train the animal to press several times to postpone the shock. Figure 11 sh the various rates of responding of a rat when eight presses are required to set the shock-timer (51). The four panels from top to bottom show the rate responding during four six-hour sessions when each lever press postponed shock for 100, 50, 20 and 15 seconds, respectively. These data show that, as in Sidman's original procedure, the rate of responding is a function of the ti period by which each response postpones the shock (33,51).

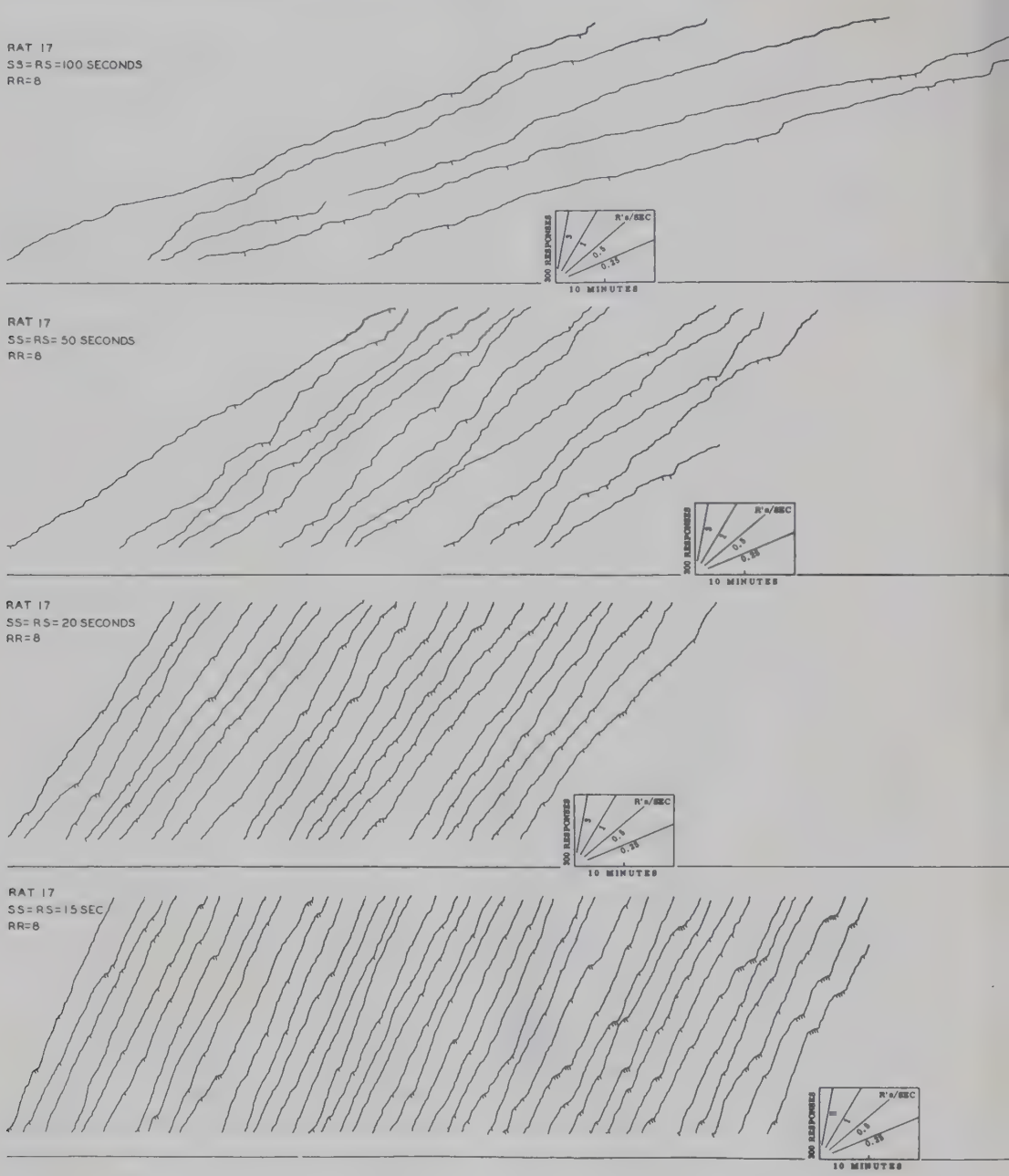

Figure 11. Performance of a rat that avoids shock by pressing a lever under a modified Sidman procedure. The performance during 4 different sessions is shown. Shocks occurred at the vertical marks on the record.

The avoidance procedures can be used very effectively to restrict an animal's repertoire and limit it almost exclusively to the emission of a single kind of behavior selected arbitrarily by the experimenter.

Williams and Teitelbaum recently employed such a technique to force satiated rats to drink exorbitantly large quantities of water (54). Similar procedures could be used to make animals overeat. The degree of control exerted by the avoidance schedules discussed here and operant-conditioning techniques in general is remarkable and frightening in its implications for human behavior. The basic contingencies involved, the postponement or reduction of an aversive consequence, are common in the daily control of human behavior (38). The ethical issues generated by the development of a science of behavior and the parallel development of a powerful behavioral technology can unfortunately not be discussed here. Relevant discussions can be found elsewhere (24,31,38,41,42).

The experimental investigation of the control over operant behavior by aversive contingencies has not been limited to animals. Hefferline in recent ingenious experiments generated pure Sidman-type avoidance behavior in humans (20). Fortunately for both experimenter and subjects, the aversive reinforcers employed were innocuous.

The subjects were relaxed in a semi-reclining chair. Recording electrodes were taped to the right hand to pick up thumb twitches. Other, dummy, electrodes were attached to various other parts of the body. The subjects were led to believe that the experimenter was interested in changes in body tension when noise was occasionally superimposed on music. During the experiment, music and noise were fed through earphones. The music consisted of semi-classical selections and the noise was a loud hum generated by a signal tracer. Music only was played during the first 10 minutes of a session during which the experimenter determined the frequency of the response before avoidance conditioning. Thereafter the noise was introduced and from then on each muscle twitch turned the noise off for 15 seconds. As in Sidman's experiments with animals, responses during the 15-minute noise-free periods reset the 15-second timer, postponing the noise for another 15-second period. Subjects conditioned rapidly, first escaping from the noise and subsequently avoiding it. Interestingly enough, when the subjects were questioned after the experiments, they showed no evidence of suspecting that they had in any way control over the noise. According to Hefferline the subjects "expressed annoyance at the noise, not only because it was unpleasant in itself, but also because it interrupted the music at times when they were particularly absorbed in it." They reported that "the situation became more tolerable after the experimenter cut down the length of the noise periods" (20). The subjects were completely unaware of their behavior!

All of the above described experiments have illustrated two kinds of consequences by means of which behavior can be established and maintained. A person who drops a coin into a Coca-Cola machine demonstrates behavior determined by a positive reinforcer. The rat that terminates electric shock by pressing a lever demonstrates behavior controlled by a negative reinforcer. Another example of the latter is provided by Chicagoans who flock to the beaches of Lake Michigan in order to escape the heat of a summer day.

On the basis of the many illustrations given so far, the reader will have little trouble in understanding the definitions of these two kinds of reinforcements. 1) The term "positive reinforcer" is a label for those behavioral consequences that increase or maintain the frequency of behavior when presented, that is when they are made contingent upon a certain bit of behavior. 2) A "negative reinforcer" is a behavioral consequence that <u>also increases</u> or maintains behavior but when <u>its removal</u> is made contingent upon a particular response (23,38). Both reinforce, i.e., they increase or maintain behavior. Because of this empirical fact the meanings of these technical terms no longer agree with

the popular concepts of reward and punishment. According to these conceptions, rewards "stamp in" behavior while punishment "stamps out" behavio The effect of punishment in the layman's sense, however, is far from simpl In agreement with our more technical definition of a negative reinforcer, it has been shown experimentally that punishment is only effective because it strengthens (conditions) competing avoidance behavior (38). The general top of punishment is a large one and the problems are complex. Because of limitations of space, these issues cannot be further discussed here.

A little reflection concerning the technical definition of a positive reinforcer gives rise to the following question: 1) What happens when we make removal of a positive reinforcer (such as money) contingent upon behavior in the case of a fine)? This particular case has been extensively investigate recently with chimpanzees and pigeons by C. B. Ferster. He demonstrated that the removal of a positive reinforcer has effects similar to those that ca be obtained by the presentation of a negative reinforcer (14,16).

In one of Ferster's experiments (14), chimpanzees were trained to pres a telephone key that occasionally, on a variable-interval schedule, produced food. The key would only pay off, however, when an overhead light was turn on. Since responding in the absence of the light was never reinforced, the chimps soon stopped pressing in its absence. The chimps were trained in such a manner that by turning the light on or off, the experimenter could start or stop the responding of the animal. After this preliminary training, red light, called the pre-time-out, or pre-aversive, stimulus, was installed next to the key. This light would come on every 15 minutes for 2-1/2 to 3 m utes. If, during the last 20 seconds of the pre-time-out period, the animal pressed the key, a one-hour time-out from the variable-interval schedule fo lowed as indicated by the absence of the overhead light. However, if the animal did not respond during the final 20 seconds of the pre-time-out warning period, no time-out would occur. The next chance for getting a long one-hou "layoff" would come again 15 minutes later when the red light came on agai

The resulting performance looked very much like the one illustrated in Figure 12. This cumulative record, however, shows the behavior of a rat or

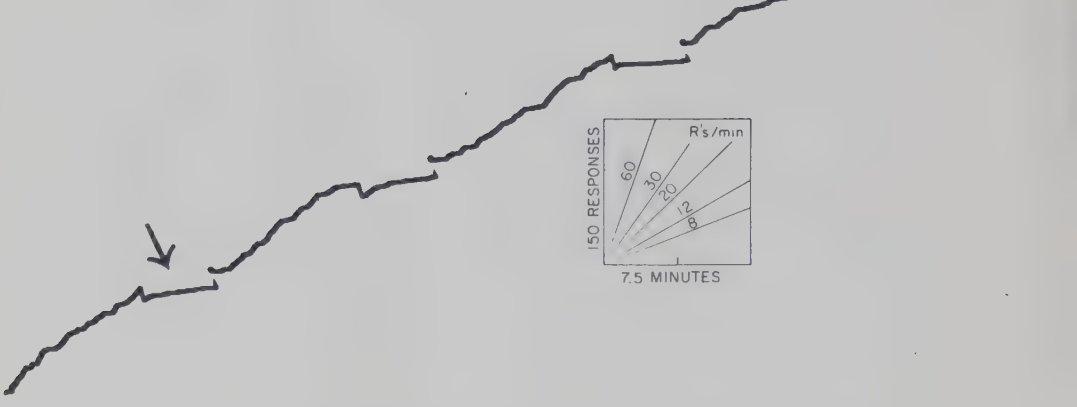

Figure 12. Suppression of lever-pressing behavior of a rat during a warning stimulus.

1-minute variable-interval schedule pressing a lever with water used as re inforcement. Every 10 minutes a tone came on for 5 minutes as indicated by the dip in the record (see arrow). An electric shock was administered to the animal unless the rat did not press the lever during the last 30 seconds of the 5-minute warning period. It is obvious from the record that the animal

ardly responded towards the end of these warning periods as indicated by ıe flattening out of the cumulative record. Data similar to those of Figure 2 were obtained by Azrin using pigeons (3).

On the basis of his experiments Ferster concluded that "The suppression f the base-line behavior by the stimulus preceding the time out from the var- able-interval schedule of reinforcement establishes the time out as an aver- ive event having properties similar to those of electric shock. The time out ıs an aversive event can be extrapolated to most aversive control in human ehavior, where noxious stimuli such as corporal punishment or electric hock are rarely used" (14).

I may mention at this point that if the reinforcement contingencies in the ast two experiments were changed in such a way that the animal had been re- uired to increase his rate of responding by a certain amount, the experimen- al situation would have been almost analogous to the conditions that confront he driver of a car at an intersection controlled by traffic lights. The amber raffic light has the same function as Ferster's pre-time-out stimulus, being ollowed after a few seconds by a red light during which the driver has to top his car. It is doubtful whether anybody would care to argue that the red ight is not aversive. The amber pre-time-out and the red time-out lights are occasionally superimposed upon the intermittently positively reinforced be- avior of driving a car. Similar to Ferster's chimpanzees, many drivers have a strong tendency to increase their rate of movement, by stepping on the gas edal, if they are close to an intersection when the yellow light appears. This often dangerous behavior could easily be eliminated by removing the amber re-time-out stimulus. A few cities recently have switched to systems in which all approaches of an intersection are simultaneously red for a certain ength of time. Whether this was done to eliminate the amber light as a pre- ime-out stimulus is doubtful.

There is an interesting opposite to the control of behavior by "time out rom positive reinforcement" (16). The author and Murray Sidman (at the Valter Reed Army Institute for Medical Research) are currently investigat- ng the case where a response is conditioned and maintained by a time out rom a stimulus associated with the avoidance schedules described previous- y. It follows from our definition of a negative reinforcer that its removal hould be rewarding.

In one of the author's experiments a certain arbitrary stimulus, for ex- mple a 433 cycle/sec. tone, is made aversive to a rat by always being asso- iated with a Sidman-type avoidance schedule. During several 6-hour experi- nental sessions, given every other day, the animal was first taught to dis- riminate between tone-on and tone-off periods, which alternated every 10 inutes. During the 10-minute tone-off period, the avoidance schedule was n effect. During the tone-on period the animal was released from the avoid- nce schedule: when the rat did not respond, he did not get shocked. Within a ew sessions under these conditions, the animal learned not to respond dur- ng the time-out but went back to work immediately when the tone went off gain. A typical performance of a rat is shown in Figure 13. In the next phase f the experiment the rat is given control over the occurrence of the time-out eriods. Pressing a second lever will produce a 10-minute time out from the hock-avoidance schedule. As is shown by the record of the animal in Figure 4, this animal produces one time out after another, spending hardly any time n the stimulus associated with the avoidance schedule.

The preceding discussion has shown that stable behavioral processes in he individual organism can be demonstrated and objectively recorded by means f operant-conditioning procedures. Because of the extensive control over be- avior obtained with these methods, it is not surprising that investigators with ifferent interests have become involved in this area. I have already suggested ome applications to what are essentially nutritional and biochemical problems

and to what are matters of concern to traffic control engineers. The existe of an operant-conditioning laboratory in an industrial pharmaceutical rese institute shows that operant-conditioning techniques have aroused considera although somewhat skeptical, interest on the part of the pharmacologist. Al though a systematic experimental analysis of the effects of drugs on behavi (psychopharmacology) is still in its infancy, promising work is currently in progress in many laboratories, both industrial and academic.

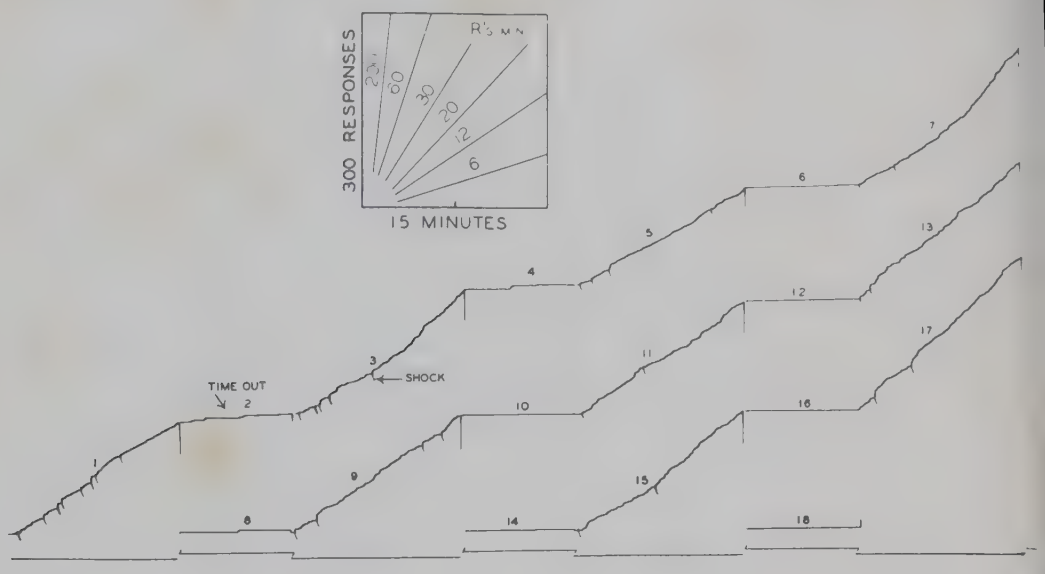

Figure 13. Performance of a rat in an avoidance procedure involving a discrimination between tone-on and tone-off periods. Tone-on periods, correlated with a time-out from the avoidance schedule, occurred during the even-numbered flat stretches in the cumulative record (baseline pen up). Shocks are indicated by the vertical marks on the record.

Since the applications of operant techniques towards an analysis of drug effects on behavior is one of my main personal interests, a short discussion seems in order. As an example of the kind of analysis to which such studies might eventually lead, let me take the effects of two well-known sympathomimetic amines that currently are being intensively investigated in several laboratories. Both methamphetamine (d-desoxyephedrine) and d-amphetamine are usually classified as central-nervous-system stimulants as well as appetite suppressants. The effects implied by these rather loose pharmacological labels can be studied quantitatively in the laboratory by means of operant-conditioning methods. The stimulating component can be demonstrated, for example, by administering one of these drugs to animals in an experimental situation that involves avoidance from shock behavior (49,50). Figure 15 shows the effect of 1 mg./kg. of methamphetamine, administered subcutaneously, on the behavior of a rat in the same experimental situation as that illustrated in Figure 13. As is demonstrated by these data, the drug at this dose produces a large increase in the rate of avoidance responding. This is, however, not the only effect: the animal also considerably increases his rate of responding in the time-out periods, possibly indicating a breakdown of stimulus control by the tone. It can further be demonstrated that the effect on such a discrimination is not specific to auditory discrimination. Identical results are obtained if a flashing light is used as the stimulus indicating a time-out. Moreover the percentage increase of the rate of responding in the avoidance periods is smaller than the percentage increase of the response-rate in the time-out p

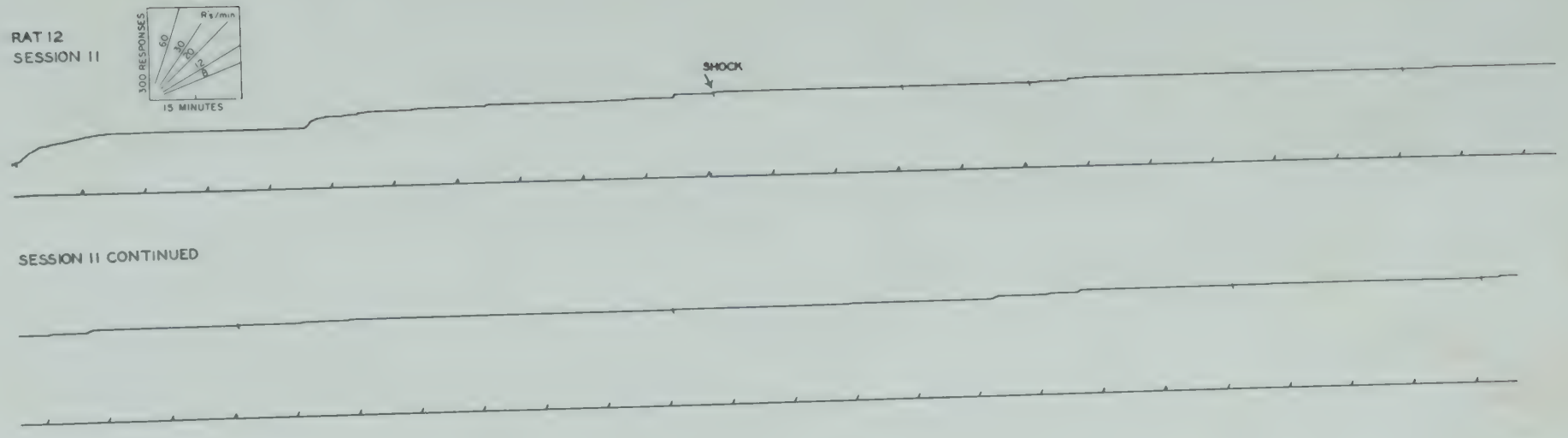

Figure 14. Performance of a rat on the avoidance lever in a procedure identical to that described for Figure 13 except that the rat can produce the time-out from the avoidance schedule himself by pressing a second lever in his cage. The time-out from the avoidance period was present whenever the baseline pen was in the down position.

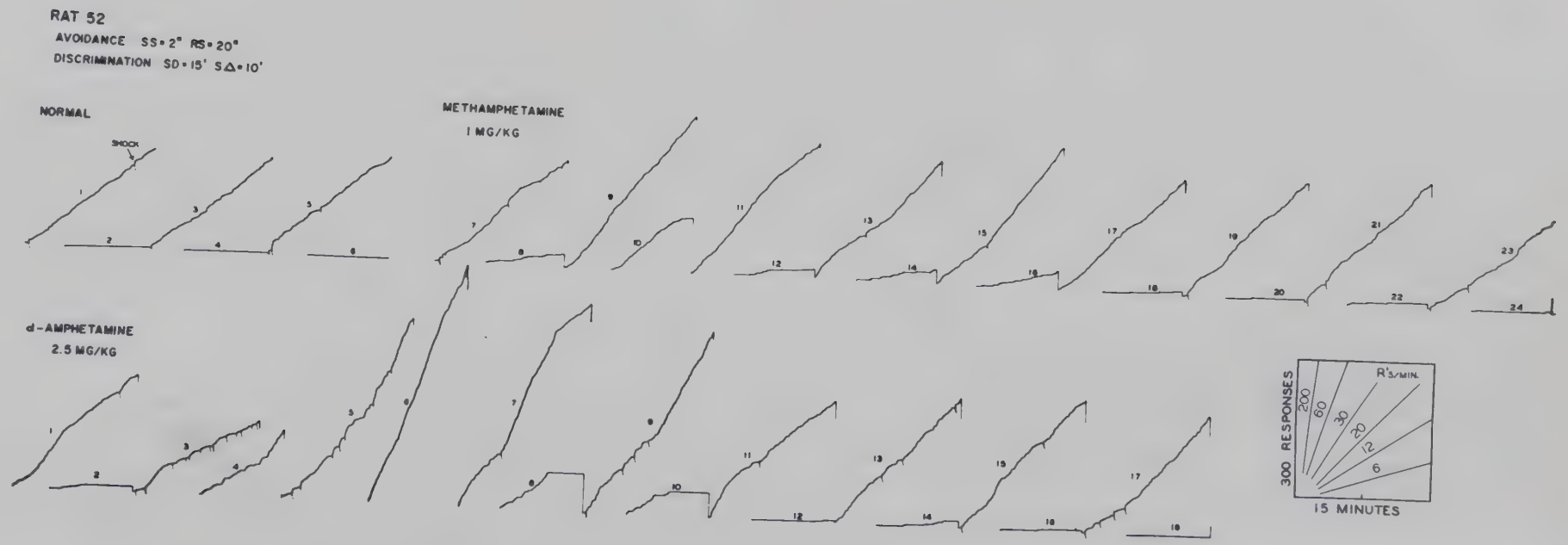

Figure 15. Effect of two sympathomimetic amines on a performance similar to the one described for Figure 13. Time-out periods from the avoidance schedule occurred during the even-numbered stretches in the record. A normal performance is shown from 1 through 6. A record obtained during a single session after injection of 1 mg./kg. of methamphetamine by subcutaneous route is shown from 7 through 24. The second panel from 1 through 18 shows the record obtained during a session after injection of 2.5 mg./kg. of d-amphetamine. The drugs were administered at the start of a session.

riods. This illustrates another aspect of the stimulating effect of both com-
pounds: the effect of these drugs is a function of the initial pre-drug baseline
control rate. The larger the initial frequency of the behavior, the smaller the
drug effect. This relationship has already been shown to have a fairly wide
generality. Dews has reported similar results with pigeons by using various
reinforcement schedules involving positive reinforcement (13).

The appetite-suppressing component can be demonstrated by administer-
ing these compounds to animals for which food is used as reinforcement. Fig-
ure 16 shows the effect of 2 mg./kg. of methamphetamine on the bar-pressing
performance of a rat that had been trained to work on a one-minute variable-
interval schedule and received one 40-milligram food pellet per reinforcement.

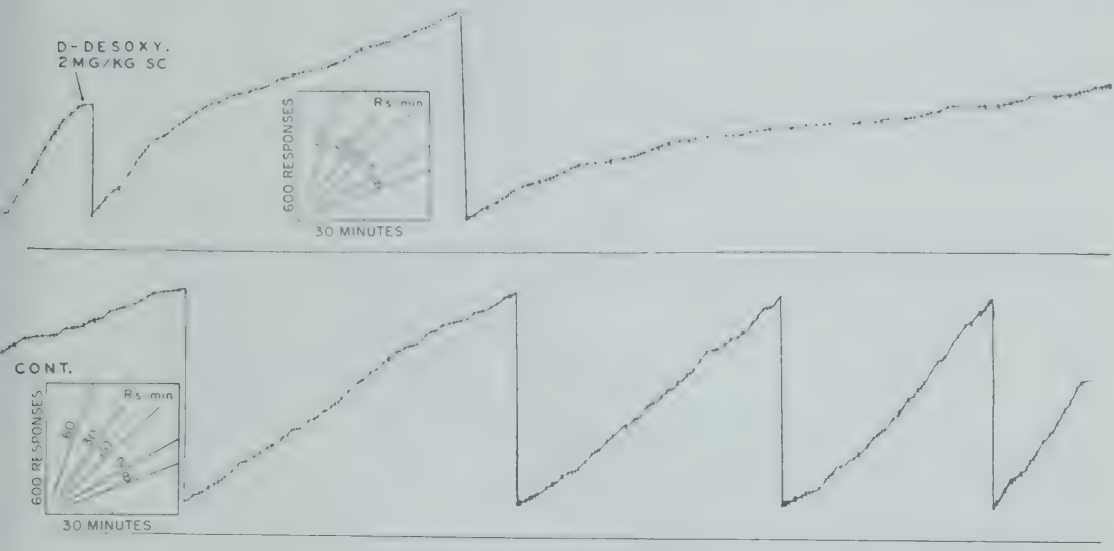

Figure 16. Performance of a rat under variable-interval reinforcement after administration of 2 mg./kg. of d-desoxy-ephedrine administered by subcutaneous route. Reinforcements occurred at the vertical marks on the record.

The record shows a depression of the response-rate almost immediately after administration of the drug. The degree of depression gradually increased during the next 90 minutes, the peak suppression of the rate lasted for almost two more hours. The drug effect subsequently wore off and the rat continued to eat his ration of several hundred pellets during the rest of the session at an almost normal pre-drug control rate. Figure 17 shows the effect of both methamphetamine and d-amphetamine on the bar-pressing performance of a rat on a small fixed-ratio schedule (FR 30). In these experiments* rats were reinforced with 1/2 cc. of sweetened condensed milk for every 30th press. The effects of several doses of each compound in the same rat during different sessions are illustrated. The outstanding drug effect in these experiments shows up as a long period immediately after drug administration during which the animal made no response. This pause is clearly a function of increasing dosage. Interestingly enough, when the animal starts responding again (always abruptly) the rate is hardly distinguishable from the normal control performance. Comparing the effects of methamphetamine in the experiments of Figures 16 and 17, we may conclude that, although in both cases these compounds depress food-reinforced behavior, the qualitative effect depends on the schedule of reinforcement employed but not on the qualitative differences of food

*These experiments were performed by John E. Owen, Jr.

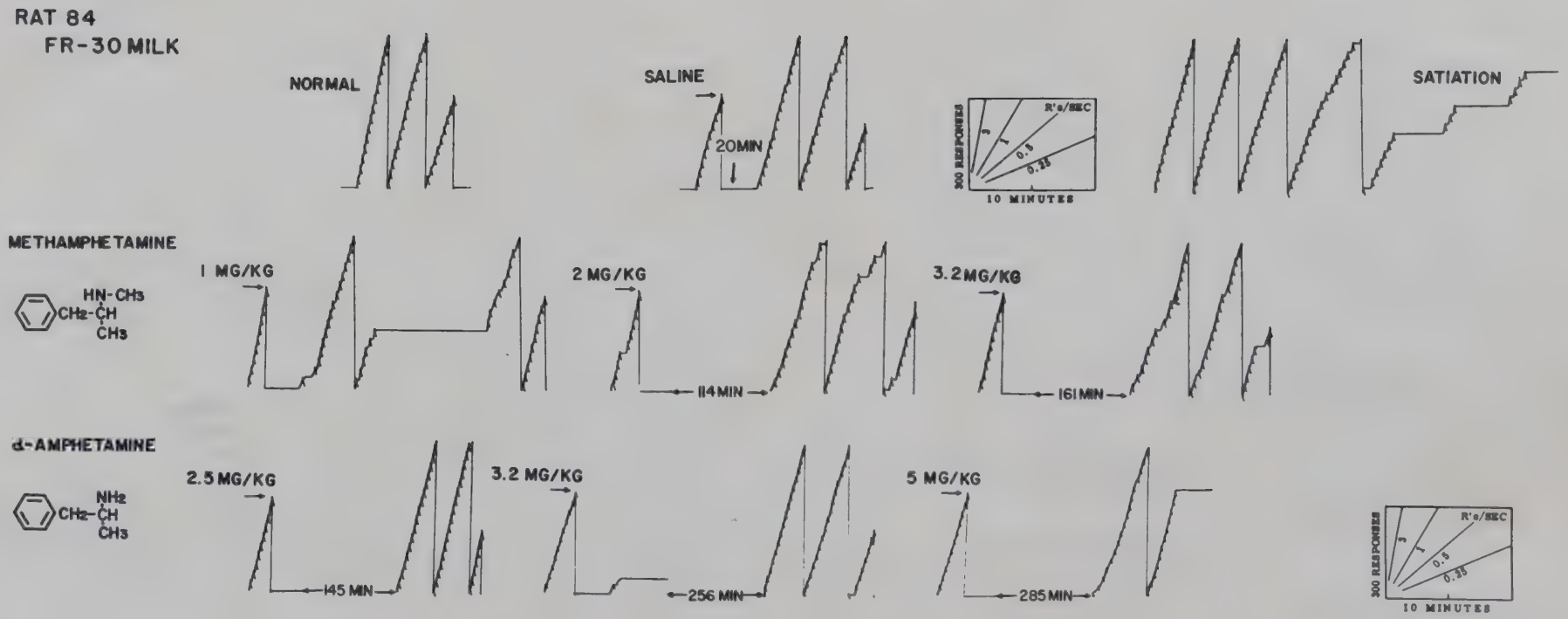

Figure 17. Performance of a rat under fixed-ratio reinforcement showing the effect of various doses of 2 sympathomimetic amines. The performance during nine different sessions is shown. The top panel shows a normal performance, one after injection of saline and a session showing the beginning of satiation. Reinforcements are indicated by vertical marks on the records.

einforcers such as milk and solid food pellets. The depressant action in both ases is especially remarkable considering the fact that in all of these exper- nents the rats were starved down to 80% of their ad. lib. feeding weight. omparing the gross effect of the drugs on food reinforced behavior with the ffects of satiation on similar schedules (Figures 7 and 8), we might conclude iat these compounds mimic to some extent the effects of the satiation vari- ble (at certain doses). Another general conclusion that can be drawn is that he effect of a drug on behavior depends on the reinforcement employed: shock voidance versus food-reinforced behavior. This last general conclusion has een confirmed by other research workers using a variety of drugs (7,13,34).

Besides pharmacology, operant-conditioning methods have been found use- ul for researches in many other areas, such as neuroanatomy (15,45), neuro- hysiology (45), anesthesiology (27), endocrinology (28), psychosomatic medi- ine (8,29), sensory physiology (5), psychophysics (6), education (40,47), lin- uistics (46), and human engineering (21,22,25). Limitation of space unfortu- ately prevents the discussion of all of this relevant material.

One more rather intriguing application of operant-conditioning methods nvolves the large-scale commercial production of animal behavior for pur- oses of television, advertising, and other industrial uses. K. Breland, a for- ner student of B. F. Skinner, has for the last ten years successfully applied he basic principles of operant behavior towards the training of animals for dvertising exhibits at county fairs, etc. (9). Many people in this country have t one time or another seen his baseball-playing chickens, tightrope walking ens, basketball-playing rabbits, or "Priscilla the Fastidious Pig" (55). Train- ng animals for such elaborate acts is no mean accomplishment, which implies great deal of technical know-how (37) unknown to the traditional circus train- r (18). Technically, they easily match the feats of the renowned Russian ani- al trainer Durov, whose methods, like Breland's, also involve the use of pos- ive reinforcement as well as the use of intermittent reinforcement sched- les (17,35).

As far as the other industrial uses alluded to at the beginning of the pre- ious paragraph are concerned, A. C. Clarke, in a half serious vein, suggest- d in a recent article in Harper's Magazine (11), that "with modern knowledge f animal psychology and conditioned reflexes, we should be able to train ome of our companions on the globe for much more sophisticated tasks" than ose involved in using dogs and falcons for hunting and elephants, horses and aks for transport. Clarke suggested training the chimpanzee for doing house- old chores. Such speculations are not entirely idle. I have recently trained igeons to inspect small objects for minor visual details. The bird was taught o look at an object automatically brought forward behind a regular transpar- nt pigeon key and to peck 2 different keys for commercially acceptable and on-acceptable objects ("scags"). A top-view of part of the apparatus used is nown in Figure 18. This photograph caught a bird in action at the moment of aking his decision which of the two keys to peck. It was shown in this experi- ent that pigeons can be reliable quality-control inspectors.

CONCLUSION

A paper of this sort can only give a bird's eye view of a large body of ata and techniques which has grown explosively during the last few years.

I have only been able to suggest the wide range of application of the oper- nt-conditioning techniques developed by Skinner and his colleagues. The ex- ent to which progress is being made towards an integrated description of the any facts generated by an experimental analysis of behavior could only be uggested.

In summary, the techniques for the experimental analysis of voluntary

Figure 18. A pigeon in an experimental chamber with two keys. Small objects are moved up one by one behind the transparent left key by means of an intermittently moving belt.

behavior described in this paper employ the frequency with which an organism performs a certain arbitrary bit of behavior as a basic datum for study. Apparatus and certain well-established experimental results were discussed. The methods described provide continuous and objective records of the rate of responding of individual subjects during extended experimental sessions. It was pointed out that accurate and extensive prediction and control of the behavior of individual organisms as diverse as rats, pigeons and chimpanzees could be achieved. Applications of operant-conditioning techniques in other fields of science like pharmacology and nutrition were discussed.

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